3. DOSIMETRY OF DIESEL EXHAUST PARTICLES IN THE RESPIRATORY TRACT

3.1. INTRODUCTION

This chapter presents the data and current scientific thought on the deposition and clearance of diesel particulate matter (DPM) from biological systems as well as discussion of the measures of DPM in tissues. The overall goal of the chapter is to address the issue of animal-to-human extrapolation by integrating these data and thoughts into an estimate of a "human equivalent concentration" (HEC), i.e., the concentration in humans corresponding to those used in animal studies. Models that codify and integrate these data and thoughts to estimate the HEC are described and evaluated. This information is needed to inform the dose-response and extrapolation analyses in Chapter 6 and to facilitate understanding of animal carcinogenicity and the bioavailability of particle organics in the lung.

The major constituents of diesel engine exhaust and their atmospheric reaction products are described in Chapter 2 and in the report on diesel exhaust issued by the Health Effects Institute (Health Effects Institute, 1995). Diesel engine exhaust consists of a complex mixture of typical combustion gases, vapors, low-molecular-weight hydrocarbons, and particles; it is the particle phase that is of greatest health concern.

Because pulmonary toxicity is the major focal point, dosimetric considerations are limited to the lung. The dosimetric aspects of DPM to be considered in this chapter include the characteristics of DPM, deposition of DPM in the conducting airways and alveolar regions, normal DPM clearance mechanisms and rates of clearance in both regions, clearance rates during lung overload, elution of organics from DPM, transport of DPM to extra-alveolar sites, and the interrelationships of these factors in
determining the target organ dose. Although assessment of dose-response relationships may permit more advanced extrapolations from high experimental exposure concentrations to ambient levels and from animal test species to humans, the question of mechanistic similarities in a tumorigenic response between rats and humans remains unanswered and the relevance of the tumorigenic response in rats to humans questionable.

3.2. CHARACTERISTICS OF INHALED DPM AND RELATIONSHIP TO $PM_{2.5}$

The formation, transport, and characteristics of DPM are considered in detail in Chapter 2 and in the report on diesel exhaust (Health Effects Institute, 1995). DPM consists of aggregates of spherical carbonaceous particles (about 0.2 μm MMAD) to which significant amounts of higher-molecular-weight organic compounds are adsorbed (Figure 2-1) as the hot engine exhaust is cooled to ambient temperature. DPM has an extremely large surface area that allows for the

adsorption of organic compounds. Typically, 10% to 40% of DPM mass consists of organic compounds (Health Effects Institute, 1995). This figure compares with mass apportionment of 20.9% to organic compounds in $PM_{2.5}$ samples collected at sites in the eastern United States (U.S. EPA, 1996). These organic chemicals include high-molecular-weight hydrocarbons such as the polyaromatic hydrocarbons (PAHs) and their derivatives. DPM also contains a sulfate component that varies with the sulfur content of the fuel. DPM in areas such as Los Angeles and Denver makes up about 7% and 10%, respectively, of the fine particulate matter (PM) fraction (Health Effects Institute, 1995; Zielinska et al., 1998). In another study of fine particulate mass concentration in southern California, the percentage apportioned to diesel exhaust was even higher, ranging from 33% in downtown Los Angeles to 14% in a suburban/rural area in California (Schauer et al., 1996).

3.3. REGIONAL DEPOSITION OF INHALED DPM

This section discusses the major factors controlling the disposition of inhaled particles. Note that disposition is defined as encompassing the processes of deposition, absorption, distribution, metabolism, and elimination. The regional deposition of particulate matter in the respiratory tract is dependent on the interaction of a number of factors, including respiratory tract anatomy (airway dimensions and branching configurations), ventilatory characteristics (breathing mode and rate, ventilatory volumes and capacities), physical processes (diffusion, sedimentation, impaction, and interception), and the physicochemical characteristics (particle size, shape, density, and electrostatic attraction) of the inhaled particles. Regional deposition of particulate material is usually expressed as deposition fraction of the total particles or mass inhaled and may be represented by the ratio of the particles or mass deposited in a specific region to the number or mass of particles inspired. The factors affecting deposition in these various regions and their importance in understanding the fate of inhaled DPM are discussed in the following sections.

It is beyond the scope of this document to present a comprehensive account of the complexities of respiratory mechanics, physiology, and toxicology, and only a brief review will be presented here. The reader is referred to publications that provide a more in-depth treatment of these topics (Weibel, 1963; Brain and Mensah, 1983; Raabe et al., 1988; Stöber et al., 1993; U.S. EPA, 1996).

The respiratory tract in both humans and experimental mammals can be divided into three regions on the basis of structure, size, and function (International Commission on Radiological Protection, 1994): the extrathoracic (ET), the tracheobronchial (TB), and the alveolar (A). In humans, inhalation can occur through the nose or mouth or both (oronasal breathing). However, many animal models used in respiratory toxicology studies are obligate nose breathers.

3.3.1. Deposition Mechanisms

This section provides an overview of the basic mechanisms by which inhaled particles deposit within the respiratory tract. Details concerning the aerosol physics that explain both how and why particle deposition occurs as well as data on total human respiratory tract deposition are presented in detail in the earlier PM Criteria Document (U.S. EPA, 1996) and will only be briefly reviewed here. For more extensive discussions of deposition processes, refer to reviews by Morrow (1966), Raabe (1982), U.S. EPA (1982), Phalen and Oldham (1983), Lippmann and Schlesinger (1984), Raabe et al. (1988), and Stöber et al. (1993).

Particles may deposit by five major mechanisms (inertial impaction, gravitational settling, Brownian diffusion, electrostatic attraction, and interception). The relative contribution of each deposition mechanism to the fraction of inhaled particles deposited varies for each region of the respiratory tract.

It is important to appreciate that these processes are not necessarily independent but may, in some instances, interact with one another such that total deposition in the respiratory tract may be less than the calculated probabilities for deposition by the individual processes (Raabe, 1982). Depending on the particle size and mass, varying degrees of deposition may occur in the extrathoracic (or nasopharyngeal), tracheobronchial, and alveolar regions of the respiratory tract.

Upon inhalation of particulate matter such as found in diesel exhaust, deposition will occur throughout the respiratory tract. Because of high airflow velocities and abrupt directional changes in the ET and TB regions, inertial impaction is a primary deposition mechanism, especially for particles $\geq$ 2.5 µm d_{ae} (aerodynamic equivalent diameter). Although inertial impaction is a prominent process for deposition of larger particles in the tracheobronchial region, it is of minimal significance as a determinant of regional deposition patterns for diesel exhaust particles, which have an $d_{ae} \leq$ 1 µm and a small aspect ratio (ratio of the length to diameter).

All aerosol particles are continuously influenced by gravity, but particles with a $d_{ae} > 0.5$ µm are affected to the greatest extent. A spherical, compact particle will acquire a terminal settling velocity when a balance is achieved between the acceleration of gravity acting on the particle and the viscous resistance of the air; it is this velocity that brings the particle into contact with airway surfaces. Both sedimentation and inertial impaction cause the deposition of many particles within the same size range. These deposition processes act together in the ET and TB regions, with inertial impaction dominating in the upper airways and sedimentation becoming increasingly dominant in the lower conducting airways, especially for the largest particles, which can penetrate into the smaller bronchial airways.

As particle diameters become <1 µm, the particles are increasingly subjected to diffusive deposition because of random bombardment by air molecules, which results in contact with airway surfaces. A d_{ae} of 0.5 µm is often considered as a boundary between diffusion and

aerodynamic (sedimentation and impaction) mechanisms of deposition. Thus, instead of having an aerodynamic equivalent diameter (d_{ae}), diffusive particles of different shapes can be related to the diffusivity of a thermodynamic equivalent size based on spherical particles (Heyder et al., 1986). Diffusive deposition of particles is favored in the A region of the respiratory tract by the proximate surfaces and by relatively long residence times for particles.

Because their d_{ae} is generally $\leq$ 1 µm, diesel exhaust particles may deposit throughout the respiratory tract. On the basis of animal data regarding the site of origin of diesel exhaust-induced tumors, particle deposition in the alveolar region may be of greatest concern relative to the carcinogenic potential of DPM and/or the adsorbed organics. However, such data for humans are not available. As discussed above, deposition by diffusion would be especially prevalent in the A region, whereas sedimentation would be less significant for such small particles.

Electrostatic precipitation is deposition related to particle charge. The electrical charge on some particles may result in an enhanced deposition over what would be expected from size alone. This is due to image charges induced on the surface of the airway by these particles, or to space-charge effects whereby repulsion of particles containing like charges results in increased migration toward the airway wall. The effect of charge on deposition is inversely proportional to particle size and airflow rate. A recent study employing hollow airway casts of the human tracheobronchial tree that assessed deposition of ultrafine (0.02 µm) and fine (0.125 µm) particles found that deposition of singly charged particles was 5-6 times that of particles having no charge, and 2-3 times that of particles at Boltzmann equilibrium (Cohen et al., 1998). This suggests that within the TB region of humans, electrostatic precipitation may be a significant deposition mechanism for ultrafine and some fine particles, the latter of which are inclusive of DPM. Thus, although electrostatic precipitation is generally a minor contributor to overall particle deposition, it may be important for DPM.

Interception is deposition by physical contact with airway surfaces and is most important for fiber deposition; interception is described in the 1996 CD.

3.3.1.1. *Biological Factors Modifying Deposition*

The available experimental deposition data in humans are commonly derived using healthy adult Caucasian males. Various factors can act to alter deposition patterns from those obtained in this group. The effects of different biological factors, including gender, age, and respiratory tract disease, on particle deposition have been reviewed previously (U.S. EPA, 1996).

The various species that serve as the basis for dose-response assessment in inhalation toxicology studies do not receive identical doses in a comparable respiratory tract region (ET, TB, or A) when exposed to the same aerosol or gas (Brain and Mensah, 1983). Such interspecies differences are important because the adverse toxic effect is likely more related to the quantitative

pattern of deposition within the respiratory tract than to the exposure concentration; this pattern determines not only the initial respiratory tract tissue dose but also the specific pathways by which the inhaled material is cleared and redistributed (Schlesinger, 1985). Differences in patterns of deposition between humans and animals have been summarized (U.S. EPA, 1996; Schlesinger et al., 1997). Such differences in initial deposition must be considered when relating biological responses obtained in laboratory animal studies to effects in humans.

The deposition of inhaled diesel particles in the respiratory tract of humans and mammalian species has been reviewed (Health Effects Institute, 1995). Schlesinger (1985) showed that physiological differences in the breathing mode for humans (nasal or oronasal breathers) and laboratory rats (obligatory nose breathers), combined with different airway geometries, resulted in significant differences in lower respiratory tract deposition for larger particles (>1 μm d_{ae}). In particular, a much lower fraction of inhaled larger particles is deposited in the alveolar region of the rat compared with humans. However, relative deposition of the much smaller diesel exhaust particles was not affected as much by the differences among species, as was demonstrated in model calculations by Xu and Yu (1987). These investigators modeled the deposition efficiency of inhaled DPM in rats, hamsters, and humans on the basis of calculations of the models of Schum and Yeh (1980) and Weibel (1963). These simulations (Figure 3-1) indicate relative deposition patterns in the lower respiratory tract (trachea = generation 1; alveoli = generation 23) and are similar among hamsters, rats, and humans. Variations in alveolar deposition of DPM over one breathing cycle in these different species were predicted to be within 30% of one another. Xu and Yu (1987) attributed this similarity to the fact that deposition of the submicron diesel particles is dominated by diffusion rather than sedimentation or impaction. Although these data assumed nose-breathing by humans, the results would not be very different for mouth-breathing because of the low filtering capacity of the nose for particles in the 0.1 to 0.5 μm range.

However, for dosimetric calculations and modeling, it would be of much greater importance to consider the actual dose deposited per unit surface area of the respiratory tract rather than the relative deposition efficiencies per lung region. Table 3-1 compares the predicted deposited doses of diesel exhaust particles inhaled in 1 min for the three species, based on the total lung volume, the surface area of all lung airways, or the surface area of the epithelium of the alveolar region only. In Table 3-1, the deposited dose, expressed as either mass/lung volume or mass/surface area(s), is lower in humans than in the two rodent species as a result of the greater respiratory exchange rate in rodents and smaller size of the rodent lung. Such differences in the

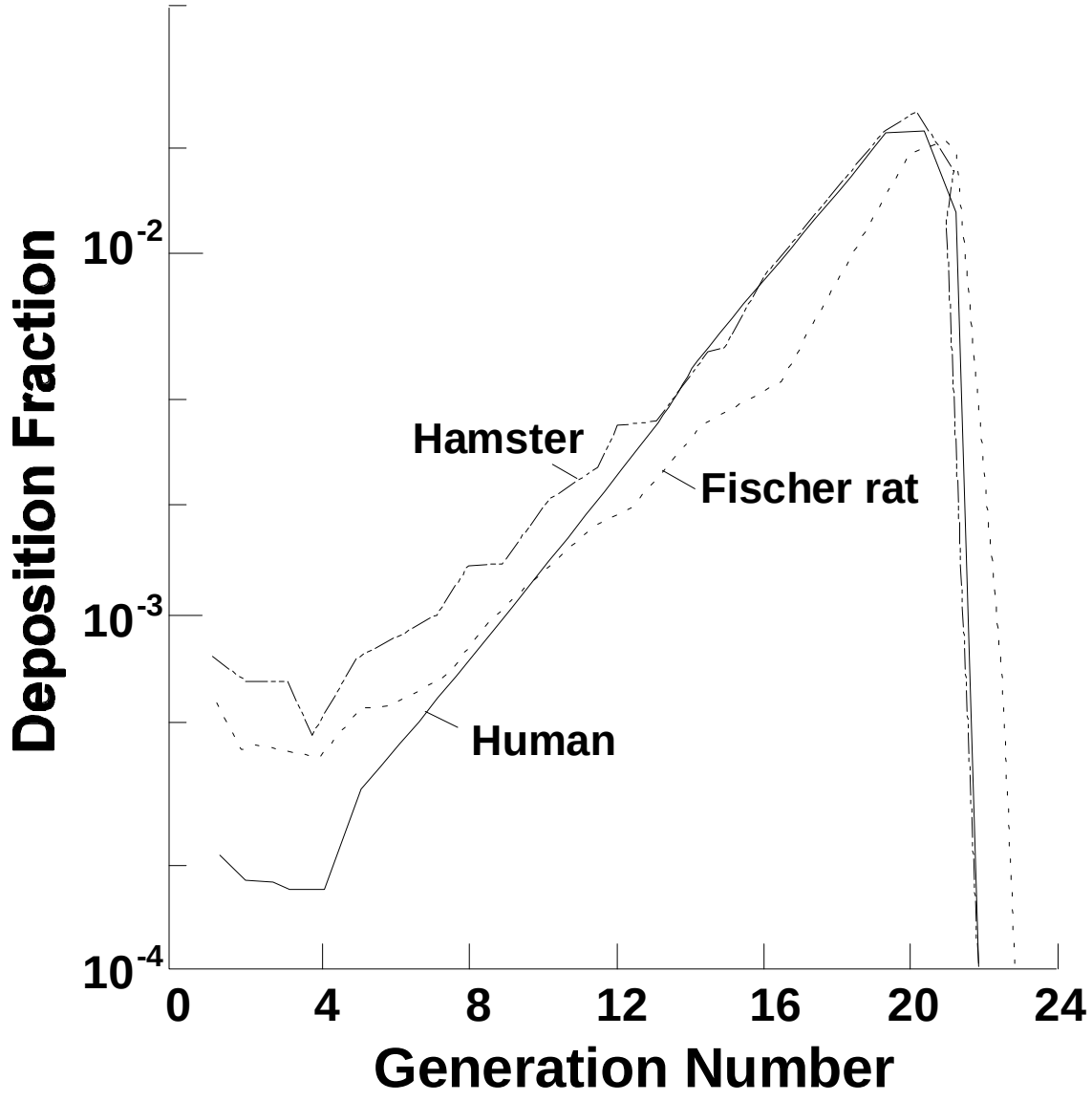

Figure 3-1. Modeled deposition distribution patterns of inhaled diesel exhaust particles in the airways of different species. Generation 1-18 are TB; >18 are A.

Source: Xu and Yu, 1987.

deposited dose in relevant target areas are important and have to be considered whenextrapolating the results from diesel exhaust exposure studies in animals to humans. Table 3-1 indicates that the differences (between humans to animals) are less on a surface area basis (≈3-fold) than on a lung volume basis (≈14-fold). This is due to larger alveolar diameters and concomitant lower surface area per unit of lung volume in humans.

Table 3-1. Predicted doses of inhaled diesel exhaust particles per minute based on total lung volume (M), total airway surface area (M_1), or surface area in alveolar region (M_2)

Species	M (10^{-3} µg/min/cm^3)	M_1 (10^{-6} µg/min/cm^2)	M_2 (10^{-6} µg/min/cm^2)
Hamster	3.548	3.088	2.382
Fischer rat	3.434	3.463	2.608
Human	0.249	1.237	0.775

$M = \frac{\text{mass DPM deposited in lung per minute}}{\text{total lung volume}}$

$M_1 = \frac{\text{mass DPM deposited in lung per minute}}{\text{total airway surface area}}$

$M_2 = \frac{\text{mass DPM deposited on the unciliated airways per minute}}{\text{surface area of the unciliated airways}}$

Based on the following conditions: (1) MMAD = 0.2 µm, σ = 1.9, ϕ = 0.3, and ρ = 1.5 g/cm^3; (2) particle concentration = 1 mg/m^3; and (3) nose-breathing.

Source: Xu and Yu, 1987.

The alternative, perhaps more accurate physiologically, is to consider deposition rate relative to exposure concentration; the deposition rate will initiate particle redistribution processes (e.g., clearance mechanisms, phagocytosis) that transfer the particles to various subcompartments, including the alveolar macrophage pool, pulmonary interstitium, and lymph nodes. Over time, therefore, only small amounts of the original particle intake would be associated with the alveolar surface.

3.3.2. Particle Clearance and Translocation Mechanisms

This section provides an overview of the mechanisms and pathways by which particles are cleared from the respiratory tract. The mechanisms of particle clearance as well as clearance routes from the various regions of the respiratory tract have been considered in the previous PM Criteria Document (U.S. EPA, 1996) and reviewed by Schlesinger et al. (1997).

Particles that deposit upon airway surfaces may be cleared from the respiratory tract completely, or may be translocated to other sites within this system, by various regionally distinct processes. These clearance mechanisms can be categorized as either absorptive (i.e., dissolution) or nonabsorptive (i.e., transport of intact particles) and may occur simultaneously or with temporal variations. Particle solubility in terms of clearance refers to solubility within the respiratory tract fluids and cells. Thus, an "insoluble" particle is one whose rate of clearance by dissolution is insignificant compared to its rate of clearance as an intact particle (as is the case

with DPM). For the most part, all deposited particles are subject to clearance by the same mechanisms, with their ultimate fate a function of deposition site, physicochemical properties (including any toxicity), and sometimes deposited mass or number concentration.

3.3.2.1. *ET Region*

The clearance of insoluble particles deposited in the nonolfactory portion of nasal passages occurs via mucociliary transport, and the general flow of mucus is backwards, i.e., towards the nasopharynx. Mucus flow in the most anterior portion of the nasal passages is forward, clearing deposited particles to the vestibular region where removal is by sneezing, wiping, or blowing.

Soluble material deposited on the nasal epithelium is accessible to underlying cells via diffusion through the mucus. Dissolved substances may be subsequently translocated into the bloodstream. The nasal passages have a rich vasculature, and uptake into the blood from this region may occur rapidly.

Clearance of poorly soluble particles deposited in the oral passages is by coughing and expectoration or by swallowing into the gastrointestinal tract.

3.3.2.2. *TB Region*

The dynamic relationship between deposition and clearance is responsible for determining lung burden at any point in time. Clearance of poorly soluble particles from the tracheobronchial region is mediated primarily by mucociliary transport and is a more rapid process than those operating in alveolar regions. Mucociliary transport (often referred to as the mucociliary escalator) is accomplished by the rhythmic beating of cilia that line the respiratory tract from the trachea through the terminal bronchioles. This movement propels the mucous layer containing deposited particles (or particles within alveolar macrophages [AMs]) toward the larynx. Clearance rate by this system is determined primarily by the flow velocity of the mucus, which is greater in the proximal airways and decreases distally. These rates also exhibit interspecies and individual variability. Considerable species-dependent variability in tracheobronchial clearance has been reported, with dogs generally having faster clearance rates than guinea pigs, rats, or rabbits (Felicetti et al., 1981). The half-time ($t_{1/2}$) values for tracheobronchial clearance of relatively insoluble particles are usually on the order of hours, as compared to alveolar clearance, which is on the order of hundreds of days in humans and dogs. The clearance of particulate matter from the tracheobronchial region is generally recognized as being biphasic or multiphasic (Raabe, 1982). Some studies have shown that particles are cleared from large, intermediate, and small airways with $t_{1/2}$ of 0.5, 2.5, and 5 h, respectively. However, reports have indicated that clearance from airways is biphasic and that the long-term component for humans may take much longer for

a significant fraction of particles deposited in this region, and may not be complete within 24 h as generally believed (Stahlhofen et al., 1990).

Although most of the particulate matter cleared from the tracheobronchial region will ultimately be swallowed, the contribution of this fraction relative to carcinogenic potential is unclear. With the exception of conditions of impaired bronchial clearance, the desorption $t_{1/2}$ for particle-associated organics is generally longer than the tracheobronchial clearance times, thereby making uncertain the importance of this fraction relative to carcinogenesis in the respiratory tract (Pepelko, 1987). Gerde et al. (1991a) showed that for low-dose exposures, particle-associated PAHs were rapidly released at the site of deposition. The relationship between the early clearance of insoluble particles (4 µm aerodynamic diameter) from the tracheobronchial regions and their longer-term clearance from the alveolar region is illustrated in Figure 3-2.

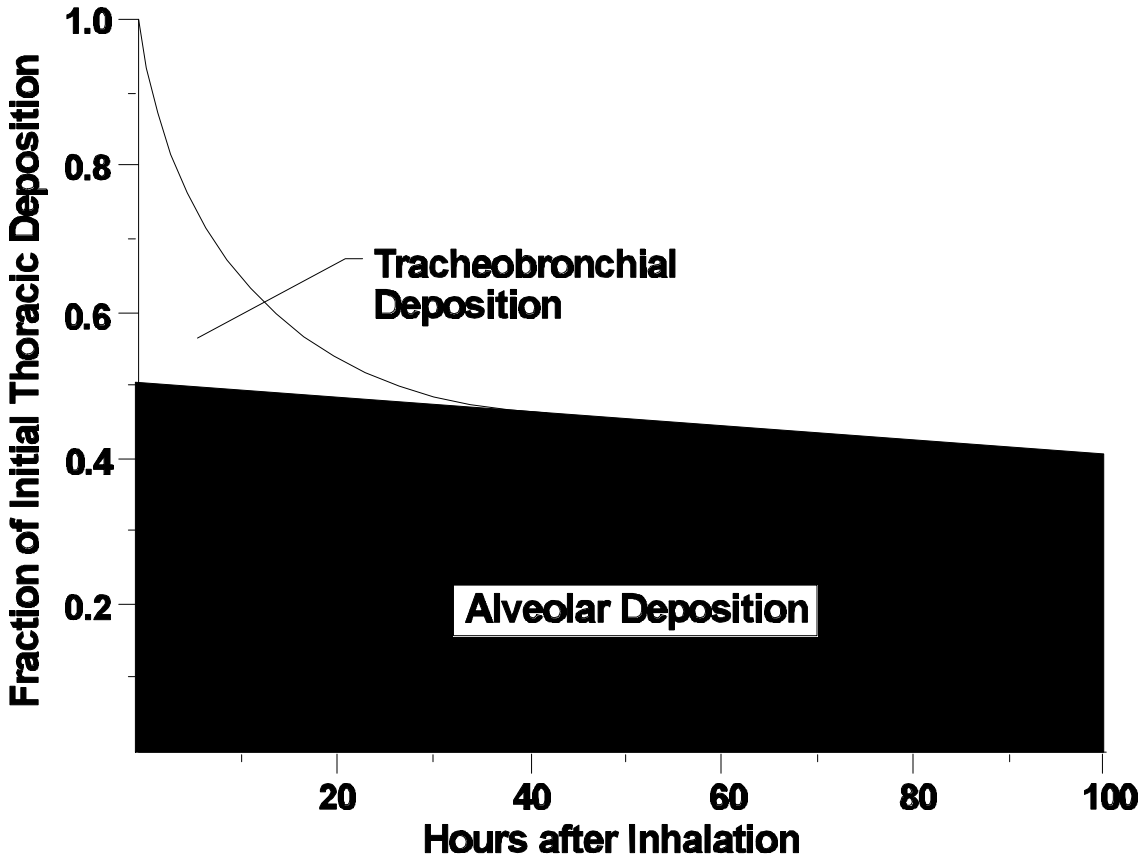

Figure 3-2. Modeled clearance of insoluble 4-µm particles deposited in tracheobronchial and alveolar regions in humans.

Source: Cuddihy and Yeh, 1986.

Cuddihy and Yeh (1986) reviewed respiratory tract clearance of particles inhaled by humans. Depending on the type of particle (ferric oxide, Teflon discs, or albumin microspheres), the technique employed, and the anatomic region (midtrachea, trachea, or main bronchi), particle velocity (moved by mucociliary transport) ranged from 2.4 to 21.5 mm/min. The highest velocities were recorded for midtracheal transport, and the lowest were for main bronchi. In one study, an age difference was noted for tracheal mucociliary transport velocity (5.8 mm/min for individuals less than 30 years of age and 10.1 mm/min for individuals over 55 years of age).

Cuddihy and Yeh (1986) described salient points to be considered when estimating particle clearance velocities from tracheobronchial regions: these include respiratory tract airway dimensions, calculated inhaled particle deposition fractions for individual airways, and thoracic (ALV + TB) clearance measurements. Predicted clearance velocities for the trachea and main bronchi were found to be similar to those experimentally determined for inhaled radiolabeled particles, but not those for intratracheally instilled particles. The velocities observed for inhalation studies were generally lower than those of instillation studies. Figure 3-3 illustrates a comparison of the short-term clearance of inhaled particles by human subjects and the model predictions for this clearance. However, tracheobronchial clearance via the mucociliary escalator is of limited importance for long-term retention.

Exposure of F344 rats to whole DPM at concentrations of 0.35, 3.5, or 7.0 mg/m^3 for up to 24 mo did not significantly alter tracheal mucociliary clearance of ^{99m}Tc-macroaggregated albumin instilled into the trachea (Wolff et al., 1987). The assessment of tracheal clearance was determined by measuring the amount of material retained 1 h after instillation. The authors stated that measuring retention would yield estimates of clearance efficiency comparable to measuring the velocity for transport of the markers in the trachea. The results of this study were in agreement with similar findings of unaltered tracheal mucociliary clearance in rats exposed to DPM (0.21, 1.0, or 4.4 mg/m^3) for up to 4 mo (Wolff and Gray, 1980). However, the 1980 study by Wolff and Gray, as well as an earlier study by Battigelli et al. (1966), showed that acute exposure to high concentrations of diesel exhaust soot (1.0 and 4.4 mg/m^3 in the study by Wolff and Gray [1980] and 8 to 17 mg/m^3 in the study by Battigelli et al. [1966]) produced transient reductions in tracheal mucociliary clearance. Battigelli et al. (1966) also noted that the compromised tracheal clearance was not observed following cessation of exhaust exposure.

The fact that tracheal clearance does not appear to be significantly impaired or is impaired only transiently following exposure to high concentrations of DPM is consistent with the absence of pathological effects in the tracheobronchial region of the respiratory tract in experimental animals exposed to DPM. However, the apparent retention of a fraction of the deposited dose in the airways is cause for some concern regarding possible carcinogenic effects in this region, especially in light of the results from simulation studies by Gerde et al. (1991b) that suggested

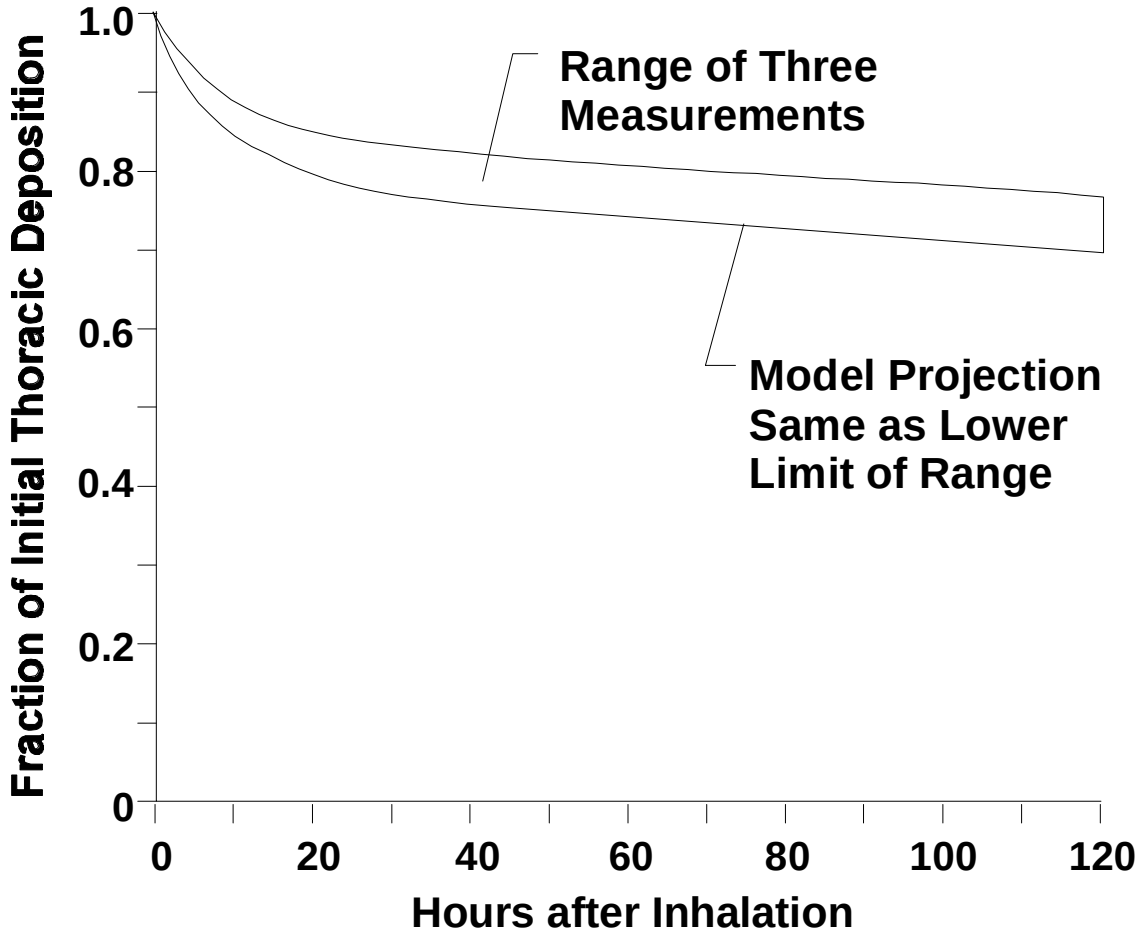

Figure 3-3. Short-term thoracic clearance of inhaled particles as determined by model prediction and experimental measurement.

Source: Cuddihy and Yeh, 1986 (from Stahlhofen et al., 1980).

that release of polycyclic aromatic hydrocarbons (PAHs) from particles may occur within minutes and at the site of initial deposition.

The absence of effects in the TB areas in long-term DPM studies and experimental evidence that particle-associated PAHs are released at the site of particle deposition together suggest that these PAHs may be of lesser importance in tumorigenic responses of rats than originally suspected. However, as noted in Section 3.6, a larger fraction of particles are deposited in the interstitium of small airways in primates than in rats (Nikula et al., 1997). Moreover, eluted PAHs are retained longer than those in the alveoli (Gerde et al., 1999), allowing time for activation. Thus PAHs may play a greater role in humans exposed to DE.

Moreover, impairment of mucociliary clearance function as a result of exposure to occupational or environmental respiratory tract toxicants or to cigarette smoke may significantly enhance the retention of particles in this region. For example, Vastag et al. (1986) demonstrated that not only smokers with clinical symptoms of bronchitis but also symptom-free smokers have significantly reduced mucociliary clearance rates. Although impaired tracheobronchial clearance could conceivably have an impact on the effects of deposited DPM in the conducting airways, it does not appear to be relevant to the epigenetic mechanism likely present in diesel exhaust-induced rat pulmonary tumors.

Poorly soluble particles (i.e., DPM) deposited within the TB region are cleared predominantly by mucociliary transport, towards the oropharynx, followed by swallowing. Poorly soluble particles may also be cleared by traversing the epithelium by endocytotic processes, and enter the peribronchial region. Clearance may occur following phagocytosis by airway macrophages, located on or beneath the mucous lining throughout the bronchial tree, or via macrophages which enter the airway lumen from the bronchial or bronchiolar mucosa (Robertson, 1980).

3.3.2.3. ***A Region***

A number of investigators have reported on the alveolar clearance kinetics of human subjects. Bohning et al. (1980) examined alveolar clearance in eight humans who had inhaled <0.4 mg of ^{85}Sr-labeled polystyrene particles (3.6 ± 1.6 μm diam.). A double-exponential model best described the clearance of the particles and provided $t_{1/2}$ values of 29 ± 19 days and 298 ± 114 days for short-term and long-term phases, respectively. It was noted that of the particles deposited in the alveolar region, 75% ± 13% were cleared via the long-term phase. Alveolar retention $t_{1/2}$ values of 330 and 420 days were reported for humans who had inhaled aluminosilicate particles of MMAD 1.9 and 6.1 μm (Bailey et al., 1982).

Quantitative data on clearance rates in humans having large lung burdens of particulate matter are lacking. Bohning et al. (1982) and Cohen et al. (1979), however, did provide evidence for slower clearance in smokers, and Freedman and Robinson (1988) reported slower clearance rates in coal miners who had mild pneumoconiosis with presumably high lung burdens of coal dust. Although information on particle burden and particle overload relationships in humans is much more limited than for experimental animal models, inhibition of clearance does seem to occur. Stöber et al. (1967) estimated a clearance $t_{1/2}$ of 4.9 years in coal miners with nil or slight silicosis, based on postmortem lung burdens. The lung burdens ranged from 2 to 50 mg/g of lung or more, well above the value for which sequestration is observed in the rat. Furthermore, impaired clearance resulting from smoking or exposure to other respiratory toxicants may

increase the possibility of an enhanced particle accumulation effect resulting from exposure to other particle sources such as diesel exhaust.

Normal alveolar clearance rates in laboratory animals exposed to DPM have been reported by a number of investigators (Table 3-2). Because the rat is the species for which experimentally induced lung cancer data are available and for which most clearance data exist, it is the species most often used for assessing human risk, and reviews of alveolar clearance studies have been generally limited to this species.

Chan et al. (1981) subjected 24 male F344 rats to nose-only inhalation of DPM (6 mg/m^3) labeled with ^{131}Ba or ^{14}C for 40 to 45 min and assessed total lung deposition, retention, and elimination. Based on radiolabel inventory, the deposition efficiency in the respiratory tract was 15% to 17%. Measurement of ^{131}Ba label in the feces during the first 4 days following exposure indicated that 40% of the deposited DPM was eliminated via mucociliary clearance. Clearance of the particles from the lower respiratory tract followed a two-phase elimination process consisting of a rapid ($t_{½}$ of 1 day) elimination by mucociliary transport and a slower ($t_{½}$ of 62 days) macrophage-mediated alveolar clearance. This study provided data for normal alveolar clearance rates of DPM not affected by prolonged exposure or particle overloading.

Several studies have investigated the effects of exposure concentration on the alveolar clearance of DPM by laboratory animals. Wolff et al. (1986, 1987) provided clearance data ($t_{½}$) and lung burden values for F344 rats exposed to diesel exhaust for 7 h/day, 5 days/week for 24 mo. Exposure concentrations of 0.35, 3.5, and 7.0 mg of DPM/m^3 were employed in this whole body-inhalation exposure experiment. Intermediate (hours-days) clearance of $^{67}Ga_2O_3$ particles (30 min, nose-only inhalation) was assessed after 6, 12, 18, and 24 mo of exposure at all of the DPM concentrations. A two-component function described the clearance of the administered radiolabel:

$$F_{(t)} = A\exp(-0.693\,t/t_1) + B\exp(-0.693\,t/t_2),$$

where $F_{(t)}$ was the percentage retained throughout the respiratory tract, *A* and *B* were the magnitudes of the two components (component *A* representing the amount cleared from nasal, lung, and gastrointestinal compartments and component *B* representing intermediate clearance from the lung compartment), and τ_1 and τ_2 were the half-times for the *A* and *B* compartments, respectively. The early retention half-times (τ_1), representing clearance from primary, ciliated conducting airways, were similar for rats in all exposure groups at all time points except for those in the high-exposure (7.0 mg/m^3) group following 24 mo of exposure, whose clearance rate was faster than that of the controls. Significantly longer *B* compartment retention half-times,

Table 3-2. Alveolar clearance in laboratory animals exposed to DPM

Species/sex	Exposure technique	Exposure duration	Particles mg/m^3	Observed effects	Reference
Rats, F-344, M	Nose only; Radiolabeled DPM	40-45 min	6	Four days after exposure, 40% of DPM eliminated by mucociliary clearance. Clearance from lower RT was in 2 phases. Rapid mucociliary ($t_{1/2}$ = 1 day; slower macrophage-mediated ($t_{1/2}$ = 62 days).	Chan et al. (1981)
Rats, F-344	Whole body; assessed effect on clearance of $^{67}Ga_2O_3$ particles	7 h/day 5 days/week 24 mo	0.35 3.5 7.0	τ_1 significantly higher with exposure to 7.0 mg/m^3 for 24 mo; τ_2 significantly longer after exposure to 7.0 mg/m^3 for 6 mo. and to 3.5 mg/m^3 for 18 mo.	Wolff et al. (1986, 1987)
Rats	Whole body	19 h/day 5 days/week 2.5 years	4	Estimated alveolar deposition = 60 mg; particle burden caused lung overload. Estimated 6-15 mg particle-bound organics deposited.	Heinrich et al. (1986)
Rats, F-344, MF	Whole body	7 h/day 5 days/week 18 mo	0.15 0.94 4.1	Long-term clearance was 87 ± 28 and 99 ± 8 days for 0.15 and 0.94 mg/m^3 groups, respectively; $t_{1/2}$ = 165 days for 4.1 mg/m^3 group.	Griffis et al. (1983)
Rats, F-344;	Nose-only; Radiolabeled ^{14}C	45 min 140 min	7 2	Rats demonstrated 3 phases of clearance with $t_{1/2}$ = 1, 6, and 80 days, representing tracheobronchial, respiratory bronchioles, and alveolar clearance, respectively. Guinea pigs demonstrated negligible alveolar clearance from day 10 to 432.	Lee et al. (1983)
Guinea pigs, Hartley		45 min	7		
Rats, F-344		20 h/day 7 days/week 7-112 days	0.25 6	Monitored rats for a year. Proposed two clearance models. Clearance depends on initial particle burden; $t_{1/2}$ increases with higher exposure. Increases in $t_{1/2}$ indicate increasing impairment of AM mobility and transition into overload condition.	Chan et al. (1984)

RT = respiratory tract.
AM = alveolar macrophage.
τ_1 = clearance from primary, ciliated airways.
τ_2 = clearance from non-ciliated passages.

representing the early clearance from nonciliated passages such as alveolar ducts and alveoli, were noted after as few as 6 mo exposure to DPM at 7.0 mg/m^3 and 18 mo exposure to 3.5 mg/m^3.

Nose-only exposures to ^{134}Cs fused aluminosilicate particles (FAP) were used to assess long-term (weeks-months) clearance. Following 24-mo exposure to DPM, long-term clearance of ^{134}Cs-FAP was significantly (p<0.01) altered in the 3.5 (cumulative exposure [C × T] of 11,760 mg·h/m^3) and 7.0 mg/m^3 C × T = 23,520 mg·h/m^3) exposure groups ($t_{½}$ of 264 and 240 days, respectively) relative to the 0.35 mg/m^3 and control groups ($t_{½}$ of 81 and 79 days, respectively). Long-term clearance represents the slow component of particle removal from the alveoli. The decreased clearance correlated with the greater particle burden in the lungs of the 3.5 and 7.0 mg/m^3 exposure groups. Based on these findings, the cumulative exposure of 11,760 mg·h/m^3 represented a particle overload condition resulting in compromised alveolar clearance mechanisms.

Heinrich et al. (1986) exposed rats 19 h/day, 5 days/week for 2.5 years to DPM at a particle concentration of about 4 mg/m^3. This is equal to a C × T of 53,200 mg·h/m^3. The deposition in the alveolar region was estimated to equal 60 mg. The lung particle burden was sufficient to result in a particle overload condition. With respect to the organic matter adsorbed onto the particles, the authors estimated that over the 2.5-year period, 6-15 mg of particle-bound organic matter had been deposited and was potentially available for biological effects. This estimation was based on the analysis of the diesel exhaust used in the experiments, values for rat ventilatory functions, and estimates of deposition and clearance.

Accumulated burden of DPM in the lungs following an 18-mo, 7 h/day, 5 days/week exposure to diesel exhaust was reported by Griffis et al. (1983). Male and female F344 rats exposed to 0.15, 0.94, or 4.1 mg DPM/m^3 were sacrificed at 1 day and 1, 5, 15, 33, and 52 weeks after exposure, and DPM was extracted from lung tissue dissolved in tetramethylammonium hydroxide. Following centrifugation and washing of the supernatant, DPM content of the tissue was quantitated using spectrophotometric techniques. The analytical procedure was verified by comparing results to recovery studies using known amounts of DPM with lungs of unexposed rats. Lung burdens were 0.035, 0.220, and 1.890 mg/g lung tissue, respectively, in rats exposed to 0.15, 0.94, and 4.1 mg DPM/m^3. Long-term retention for the 0.15 and 0.94 mg/m^3 groups had estimated half-times of 87 ± 28 and 99 ± 8 days, respectively. The retention $t_{1/2}$ for the 4.1-mg/m^3 exposure group was 165 ± 8 days, which was significantly (p<0.0001) greater than those of the lower exposure groups. The 18-mo exposures to 0.15 or 0.96 mg/m^3 levels of DPM C × T equivalent of 378 and 2,368 mg·h/m^3, respectively) did not affect clearance rates, whereas the exposure to the 4.1 mg/m^3 concentration C × T = 10,332 mg·h/m^3) resulted in impaired clearance.

In a subsequent study (Lee et al., 1983), a three-phase model was used to describe the clearance of DPM (7 mg/m^3 for 45 min or 2 mg/m^3 for 140 min) by F344 rats (24 per group) exposed by nose-only inhalation with no apparent particle overload in the lungs. The exposure protocols provided comparable total doses based on a ^{14}C radiolabel. $^{14}CO_2$ resulting from combustion of ^{14}C-labeled diesel fuel was removed by a diffusion scrubber to avoid erroneous assessment of ^{14}C intake by the animals. Retention of the radiolabeled particles was determined up to 335 days after exposure and resulted in the derivation of a three-phase clearance of the particles. The resulting retention $t_{1/2}$ values for the three phases were 1, 6, and 80 days. The three clearance phases are taken to represent removal of tracheobronchial deposits by the mucociliary escalator, removal of particles deposited in the respiratory bronchioles, and alveolar clearance, respectively. Species variability in clearance of DPM was also demonstrated because the Hartley guinea pigs exhibited negligible alveolar clearance from day 10 to day 432 following a 45-min exposure to a DPM concentration of 7 mg/m^3. Initial deposition efficiency (20% ± 2%) and short-term clearance were, however, similar to those for rats.

Lung clearance in male F344 rats preexposed to DPM at 0.25 or 6 mg/m^3 20 h/day, 7 days/week for periods lasting from 7 to 112 days was studied by Chan et al. (1984). Following this preexposure protocol, rats were subjected to 45-min nose-only exposure to ^{14}C-diesel exhaust, and alveolar clearance of radiolabel was monitored for up to 1 year. Two models were proposed: a normal biphasic clearance model and a modified lung retention model that included a slow-clearing residual component to account for sequestered aggregates of macrophages. The first model described a first-order clearance for two compartments: $R(t) = Ae^{-u1t} + Be^{-u2t}$. This yielded clearance $t_{1/2}$ values of 166 and 562 days for rats preexposed to 6.0 mg/m^3 for 7 and 62 days, respectively. These values were significantly ($p<0.05$) greater than the retention $t_{1/2}$ of 77 ± 17 days for control rats. The same retention values for rats of the 0.25 mg/m^3 groups were 90 ± 14 and 92 ± 15 days, respectively, for 52- and 112-day exposures and were not significantly different from controls. The two-compartment model represents overall clearance of the tracer particles, even if some of the particles were sequestered in particle-laden macrophages with substantially slower clearance rates. For the second model, which excluded transport of the residual fractions in sequestered macrophage aggregates, slower clearance was observed in the group with a lung burden of 6.5 mg, and no clearance was observed in the 11.8 mg group. Clearance was shown to be dependent on the initial burden of particles and, therefore, the clearance $t_{1/2}$ would increase in higher exposure scenarios. This study emphasizes the importance of particle overloading of the lung and the ramifications on clearance of particles; the significant increases in half-times indicate an increasing impairment of the alveolar macrophage mobility and subsequent transition into an overload condition. Based on these data, a particle overload effect

was demonstrated for both the high and low exposure levels (equivalent to C × T dose of 840 [transitional overload] and 7,440 mg·h/m^3).

Long-term alveolar clearance rates of particles in various laboratory animals and humans have been reviewed by Pepelko (1987). Although retention $t_{1/2}$ varies both among and within species and is also dependent on the physicochemical properties of the inhaled particles, the retention $t_{1/2}$ for humans is much longer (>8 mo) than the average retention $t_{1/2}$ of 60 days for rats.

Clearance from the A region occurs via a number of mechanisms and pathways, but the relative importance of each is not always certain and may vary between species. Particle removal by macrophages comprises the main nonabsorptive clearance process in this region. Alveolar macrophages reside on the epithelium, where they phagocytize and transport deposited material, which they contact by random motion or via directed migration under the influence of local chemotactic factors (Warheit et al., 1988).

Particle-laden macrophages may be cleared from the A region along a number of pathways described in the 1996 CD. Uningested particles or macrophages in the interstitium may traverse the alveolar-capillary endothelium, directly entering the blood (Raabe, 1982; Holt, 1981); endocytosis by endothelial cells followed by exocytosis into the vessel lumen seems, however, to be restricted to particles <0.1 μm diameter, and may increase with increasing lung burden (Lee et al., 1985; Oberdörster, 1988). Once in the systemic circulation, transmigrated macrophages, as well as uningested particles, can travel to extrapulmonary organs.

Alveolar macrophages constitute an important first-line cellular defense mechanism against inhaled particles that deposit in the alveolar region of the lung. It is well established that a host of diverse materials, including DPM, are phagocytized by AMs shortly after deposition (White and Garg, 1981; Lehnert and Morrow, 1985) and that such cell-contained particles are generally rapidly sequestered from both the extracellular fluid lining in the alveolar region and the potentially sensitive alveolar epithelial cells. In addition to this role in compartmentalizing particles from other lung constituents, AMs are prominently involved in mediating the clearance of relatively insoluble particles from the air spaces (Lehnert and Morrow, 1985). Although the details of the actual process have not been delineated, AMs with their particle burdens gain access and become coupled to the mucociliary escalator and are subsequently transported from the lung via the conducting airways. Although circumstantial, numerous lines of evidence indicate that such AM-mediated particle clearance is the predominant mechanism by which relatively insoluble particles are removed from the lungs (Gibb and Morrow, 1962; Ferin, 1982; Harmsen et al., 1985; Lehnert and Morrow, 1985; Powdrill et al., 1989).

The removal characteristics for particles deposited in alveolar region of the lung have been descriptively represented by numerous investigators as a multicompartment or multicomponent process in which each component follows simple first-order kinetics (Snipes and Clem, 1981;

Snipes et al., 1988; Lee et al., 1983). Although the various compartments can be described mathematically, the actual physiologic mechanisms determining these differing clearance rates have not been well characterized.

Lehnert (1988, 1989) performed studies using laboratory rats to examine particle-AM relationships over the course of alveolar clearance of low to high lung burdens of noncytotoxic microspheres (2.13 μm diam.) to obtain information on potential AM-related mechanisms that form the underlying bases for kinetic patterns of alveolar clearance as a function of particle lung burdens. The intratracheally instilled lung burdens varied from 1.6×10^7 particles (about 85 μg) for the low lung burden to 2.0×10^8 particles (about 1.06 mg) for the mid-dose and 6.8×10^8 particles (about 3.6 mg) for the highest lung burden. The lungs were lavaged at various times postexposure and the numbers of spheres in each macrophage counted. Although such experiments provide information regarding the response of the lung to particulate matter, intratracheal instillation is not likely to result in the same depositional characteristics as inhalation of particles. Therefore, it is unlikely that the response of alveolar macrophages to these different depositional characteristics will be quantitatively similar.

The $t_{1/2}$ values of both the early and later components of clearance were virtually identical following deposition of the low and medium lung burdens. For the highest lung burden, significant prolongations were found in both the early, more rapid, as well as the slower component of alveolar clearance. The percentages of the particle burden associated with the earlier and later components, however, were similar to those of the lesser lung burdens. On the basis of the data, the authors concluded that translocation of AMs from alveolar spaces by way of the conducting airways is fundamentally influenced by the particle burden of the cells so translocated. In the case of particle overload that occurred at the highest lung burden, the translocation of AMs with the heaviest cellular burdens of particles (i.e., greater than about 100 microspheres per AM) was definitely compromised.

On the other hand, analysis of the disappearance of AMs with various numbers of particles indicates that the particles may not exclusively reflect the translocation of AMs from the lung. The observations are also consistent with a gradual redistribution of retained particles among the AMs in the lung concurrent with the removal of particle-containing AMs via the conducting airways. Experimental support suggestive of potential processes for such particle redistribution comes from a variety of investigations involving AMs and other endocytic cells (Heppleston and Young, 1973; Evans et al., 1986; Aronson, 1963; Sandusky et al., 1977; Heppleston, 1961; Riley and Dean, 1978).

3.3.3. Translocations of Particles to Extra-alveolar Macrophage Compartment Sites

Although the phagocytosis of particles by lung-free cells and the mucociliary clearance of the cells with their particulate matter burdens represent the most prominent mechanisms that govern the fate of particles deposited in the alveolar region, other mechanisms exist that can affect both the retention characteristics of relatively insoluble particles in the lung and the lung clearance pathways for the particles. One mechanism is endocytosis of particles by alveolar lining (Type I) cells (Sorokin and Brain, 1975; Adamson and Bowden, 1978, 1981) that normally provide >90% of the cell surface of the alveoli in the lungs of a variety of mammalian species (Crapo et al., 1983). This process may be related to the size of the particles that deposit in the lungs and the numbers of particles that are deposited. Adamson and Bowden (1981) found that with increasing loads of carbon particles (0.03 μm diam.) instilled in the lungs of mice, more free particles were observed in the alveoli within a few days. The relative abundance of particles endocytosed by Type I cells also increased with increasing lung burdens of the particles, but instillation of large particles (1.0 μm) rarely resulted in their undergoing endocytosis. A 4 mg burden of 0.1 μm diameter latex particles is equivalent to 8×10^{12} particles, whereas a 4 mg burden of 1.0 μm particles is composed of 8×10^{9} particles. Regardless, DPM with volume median diameters between 0.05 and 0.3 μm (Frey and Corn, 1967; Kittleson et al., 1978) would be expected to be within the size range for engulfment by Type I cells should suitable encounters occur. Indeed, it has been demonstrated that DPM is endocytosed by Type I cells in vivo (White and Garg, 1981).

Unfortunately, information on the kinetics of particle engulfment (endocytosis) by Type I cells relative to that by AMs is scanty. Even when relatively low burdens of particulate matter are deposited in the lungs, some fraction of the particles usually appears in the regional lymph nodes (Ferin and Fieldstein, 1978; Lehnert, 1989). As will be discussed, endocytosis of particles by Type I cells is an initial, early step in the passage of particles to the lymph nodes. Assuming particle phagocytosis is not sufficiently rapid or perfectly efficient, increasing numbers of particles would be expected to gain entry into the Type I epithelial cell compartment during chronic aerosol exposures. Additionally, if particles are released on a continual basis by AMs that initially sequestered them after lung deposition, some fraction of the "free" particles so released could also undergo passage from the alveolar space into Type I cells.

The endocytosis of particles by Type I cells represents only the initial stage of a process that can lead to the accumulation of particles in the lung's interstitial compartment and the subsequent translocation of particles to the regional lymph nodes. As shown by Adamson and Bowden (1981), a vesicular transport mechanism in the Type I cell can transfer particles from the air surface of the alveolar epithelium into the lung's interstitium, where particles may be phagocytized by interstitial macrophages or remain in a "free" state for a poorly defined period

that may be dependent on the physicochemical characteristics of the particle. The lung's interstitial compartment, accordingly, represents an anatomical site for the retention of particles in the lung. Whether or not AMs, and perhaps polymorphonuclear neutrophils (PMNs) that have gained access to the alveolar space compartment and phagocytize particles there, also contribute to the particle translocation process into the lung's interstitium remains a controversial issue. Evidence that such migration of AMs may contribute to the passage of particles to the interstitial compartment and also may be involved in the subsequent translocation of particles to draining lymph nodes has been obtained with the dog model (Harmsen et al., 1985).

The fate of particles once they enter the lung's interstitial spaces remains unclear. Some particles, as previously indicated, are phagocytized by interstitial macrophages, whereas others apparently remain in a free state in the interstitium for some time without being engulfed by interstitial macrophages. It is unknown if interstitial macrophages subsequently enter the alveoli with their engulfed burdens of particles and thereby contribute to the size of the resident AM population over the course of lung clearance. Moreover, no investigations have been conducted to date to assess the influence that the burden of particles may have on the ability of the interstitial macrophage to migrate into the alveolar space compartment.

At least some particles that gain entry into the interstitial compartment can further translocate to the extrapulmonary regional lymph nodes. This process apparently can involve the passage of free particles as well as particle-containing cells via lymphatic channels in the lungs (Harmsen et al., 1985; Ferin and Fieldstein, 1978; Lee et al., 1985). It is conceivable that the mobility of the interstitial macrophages could be particle-burden limited, and under conditions of high cellular burdens a greater fraction of particles that accumulate in the lymph may reach these sites as free particles. Whatever the process, existing evidence indicates that when lung burdens of particles result in a particle-overload condition, particles accumulate both more rapidly and abundantly in lymph nodes that receive lymphatic drainage from the lung (Ferin and Feldstein, 1978; Lee et al., 1985).

3.3.3.1. *Clearance Kinetics*

The clearance kinetics of PM have been reviewed in the PM CD (U.S. EPA, 1996) and by Schlesinger et al. (1997). Deposited particles may be completely or incompletely cleared from the respiratory tract. However, the time frame over which clearance occurs affects the cumulative dose delivered to the respiratory tract, as well as to extrapulmonary organs.

3.3.3.2. *Interspecies Patterns of Clearance*

The inability to study the retention of certain materials in humans for direct risk assessment requires the use of laboratory animals. Since dosimetry depends on clearance rates

and routes, adequate toxicological assessment necessitates that clearance kinetics in these animals be related to those in humans. The basic mechanisms and overall patterns of clearance from the respiratory tract are similar in humans and most other mammals. However, regional clearance rates can show substantial variation between species, even for similar particles deposited under comparable exposure conditions. This has been extensively reviewed in the previous document (U.S. EPA, 1996) and in other papers (Schlesinger et al., 1997; Snipes et al., 1989).

In general, there are species-dependent rate constants for various clearance pathways. Differences in regional and total clearance rates between some species are a reflection of differences in mechanical clearance processes. For consideration in assessing particle dosimetry, the end result of interspecies differences in clearance is that the retention of deposited particles can differ between species, which may result in differences in response to similar particulate exposure atmospheres.

3.3.3.3. *Biological Factors Modifying Clearance*

A number of host and environmental factors may modify normal clearance patterns. These include age, gender, physical activity, respiratory tract disease, and irritant inhalation (U.S. EPA, 1996).

3.3.3.4. *Respiratory Tract Disease*

Earlier studies reviewed in the PM CD (U.S. EPA, 1996) noted that various respiratory tract diseases are associated with clearance alterations. The examination of clearance in individuals with lung disease requires careful interpretation of results, since differences in deposition of tracer particles used to assess clearance function may occur between normal individuals and those with respiratory disease, and this would directly impact upon the measured clearance rates, especially in the tracheobronchial tree. Prolonged nasal mucociliary clearance in humans is associated with chronic sinusitis, bronchiectasis or rhinitis, and cystic fibrosis. Bronchial mucus transport may be impaired in people with bronchial carcinoma, chronic bronchitis, asthma, and various acute infections. In certain of these cases, coughing may enhance mucus clearance, but it generally is effective only if excess secretions are present.

The rates of A region particle clearance were reduced in humans with chronic obstructive lung disease and in laboratory animals with viral infections, while the viability and functional activity of macrophages were impaired in human asthmatics and in animals with viral-induced lung infections (U.S. EPA, 1996). However, any modification of functional properties of macrophages appears to be injury specific, reflecting the nature and anatomic pattern of disease.

3.4. PARTICLE OVERLOAD

3.4.1. Introduction

Some experimental studies using laboratory rodents employed high exposure concentrations of relatively nontoxic, poorly soluble particles. These particle loads interfered with normal clearance mechanisms, producing clearance rates different from those that would occur at lower exposure levels. Prolonged exposure to high particle concentrations is associated with what is termed particle overload. This is defined as the overwhelming of macrophage-mediated clearance by the deposition of particles at a rate exceeding the capacity of that clearance pathway.

Wolff et al. (1987) used ^{134}Cs-labeled fused aluminosilicate particles to measure alveolar clearance in rats following 24-mo exposure to low (L), medium (M), and high (H) concentrations of diesel exhaust (targeted concentrations of DPM of 0.35, 3.5 and 7.0 mg/m^3). The short-term component of the multicomponent clearance curves was similar for all groups, but long-term clearance was retarded in the M and H exposure groups (Figure 3-4). The half times of the long-term clearance curves were 79, 81, 264, and 240 days, respectively, for the control, L, M, and The observed lung burdens increased progressively, reaching levels of 11.5 and 20.5 mg H exposure groups. Clearance was overloaded at M and H exposure levels, but not by the

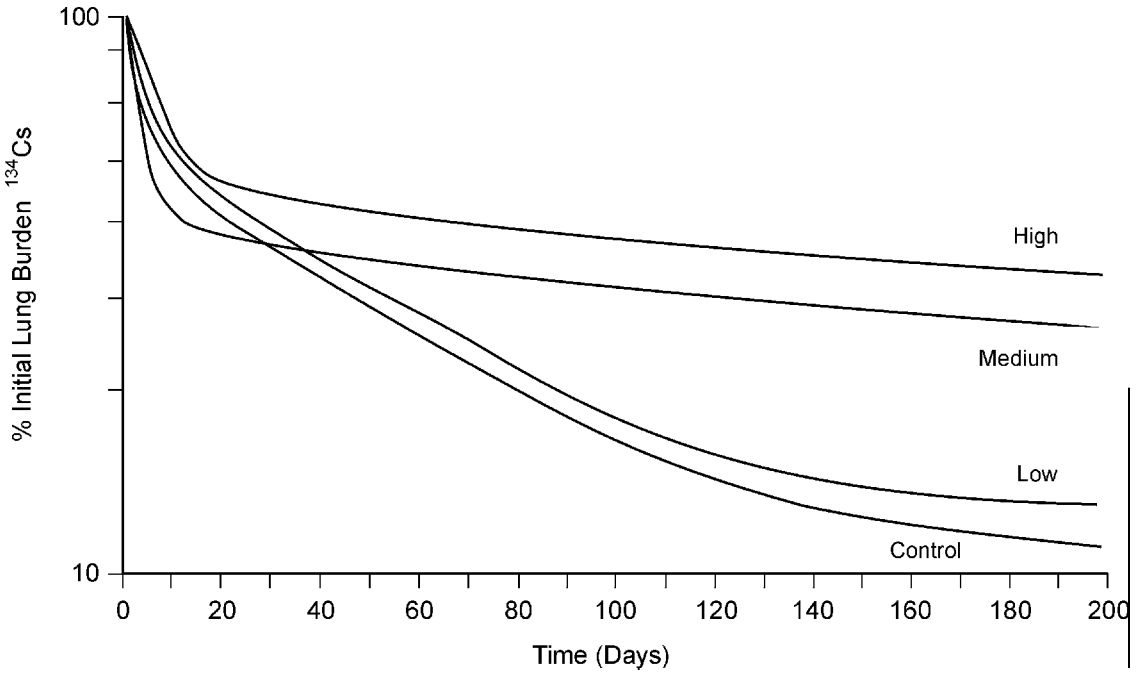

Figure 3-4. Clearance from lungs of rats of ^{134}Cs-FAP fused aluminosilicate tracer particles inhaled after 24 months of diesel exhaust exposure at concentrations of 0 (control) (●), 0.35 (low) (■), 3.5 (medium) (●), and 7.0 (high) mg DPM/m^3 (▲). Points on curves are means ± SE.

Source: Wolff et al., 1987.

L exposure level. Lung burdens of DPM were measured after 6, 12, 18, and 24 mo of exposure. DPM/lung, respectively, after 24 mo in the M and H exposed groups (Figure 3-5). The results indicate that the clearance of freshly deposited particles was retarded after 24 mo of DPM exposure at the two highest exposure levels, and that clearance had become overloaded at these two exposures but not at the lowest exposure.

It has been hypothesized that overloading will begin in the rat when deposition approaches 1 mg particles/g lung tissue (Morrow, 1988). When the concentration reaches 10 mg particles/g lung tissue, macrophage-mediated clearance of particles would effectively cease. It is a nonspecific effect noted in experimental studies, generally in rats, using many different kinds of poorly soluble particles (including TiO_2, volcanic ash, DPM, carbon black, and fly ash) and results in A region clearance slowing or stasis, with an associated inflammation and aggregation of macrophages in the lungs and increased translocation of particles into the interstitium (Muhle et al., 1990; Lehnert, 1990; Morrow, 1994). Following overloading, the subsequent retardation

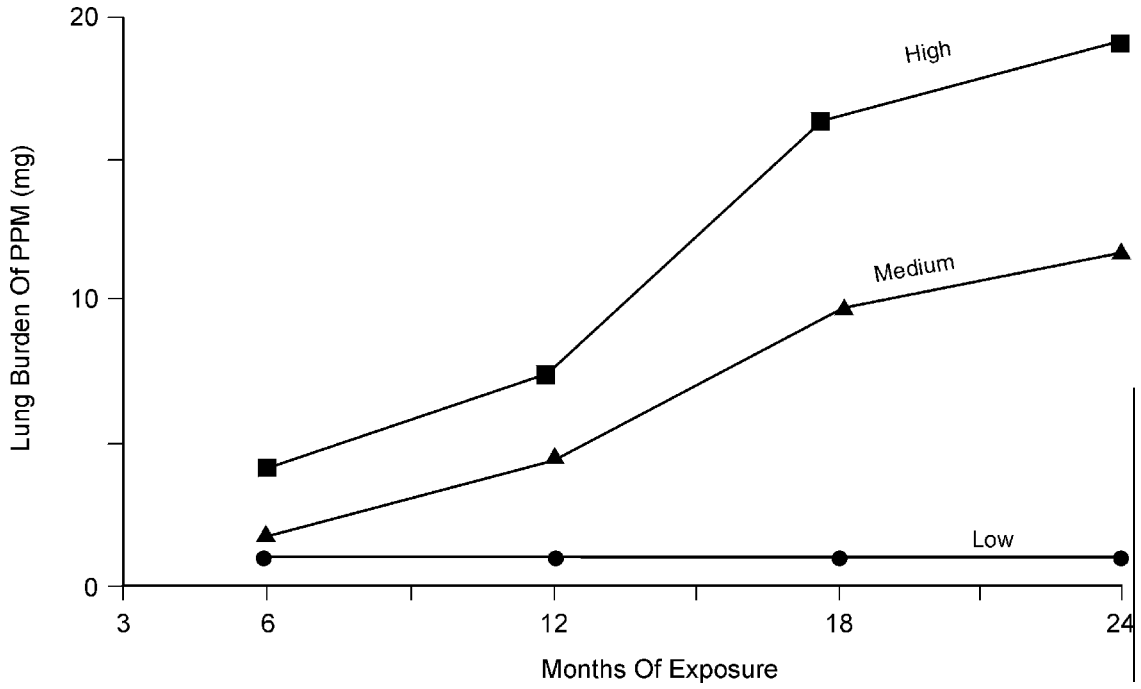

Figure 3-5. Lung burdens of DPM within rats exposed to 0.35 (low) (●), 3.5 (medium) (▲), and 7.0 (high) mg ppm/m^3 (■).

Source: Wolff et al., 1987.

of lung clearance, accumulation of particles, inflammation, and the interaction of inflammatory mediators with cell proliferative processes and DNA may lead to the development of tumors and fibrosis in rats (Mauderly, 1996). The phenomenon of overload has been discussed in greater detail in the previous PM CD (U.S. EPA, 1996).

3.4.2. Relevance to Humans

The relevance of lung overload to humans, and even to species other than laboratory rats and mice, is not clear. While it is likely to be of little relevance for most "real world" ambient exposures of humans, it is of concern in interpreting some long-term experimental exposure data and perhaps for humans' occupational exposure. In addition, relevance to humans is clouded by the suggestion that macrophage-mediated clearance is normally slower and perhaps less important in humans than in rats (Morrow, 1994), and that there can be significant differences in macrophage loading between species.

Particle overload appears to be an important factor in the diesel emission-induced pulmonary carcinogenicity observed in rats. Studies described in this section provide additional data showing a particle overload effect. A study by Griffis et al. (1983) demonstrated that exposure (7 h/day, 5 days/week) of rats to DPM at concentrations of 0.15, 0.94, or 4.1 mg/m^3 for 18 mo resulted in lung burdens of 0.035, 0.220, and 1.890 mg/g of lung tissue, respectively. The alveolar clearance of those rats with the highest lung burden (1.890 mg/g of lung) was impaired, as determined by a significantly greater (p<0.0001) retention $t_{1/2}$ for DPM. This is reflected in the greater lung burden/exposure concentration ratio at the highest exposure level. Similarly, in the study by Chan et al. (1984), rats exposed for 20 h/day, 7 days/week to DPM (6 mg/m^3) for 112 days had a total lung particle burden of 11.8 mg, with no alveolar particle clearance being detected over 1 year.

Muhle et al. (1990) indicated that overloading of rat lungs occurred when lung particle burdens reached 0.5 to 1.5 mg/g of lung tissue and that clearance mechanisms were totally compromised at lung particle burdens $\geq$ 10 mg/g for particles with a specific density close to 1.

Pritchard (1989), utilizing data from a number of diesel exhaust exposure studies, examined alveolar clearance in rats as a function of cumulative exposure. The resulting analysis noted a significant increase in retention $t_{1/2}$ values at exposures above 10 mg/m^3·h/day and also showed that normal lung clearance mechanisms appeared to be compromised as the lung DPM burden approached 0.5 mg/g of lung.

Morrow (1988) has proposed that the condition of particle overloading in the lungs is caused by a loss in the mobility of particle-engorged AMs and that such an impediment is related to the cumulative volumetric load of particles in the AMs. Morrow (1988) has further estimated that the clearance function of an AM may be completely impaired when the particle burden in the AM is of a volumetric size equivalent to about 60% of the normal volume of the AM. Morrow's

hypothesis was the initial basis for the physiology-oriented multicompartmental kinetic (POCK) model derived by Stöber et al. (1989) for estimating alveolar clearance and retention of biologically insoluble, respirable particles.

A revised version of this model refines the characterization of the macrophage pool by including both the mobile and immobilized macrophages (Stöber et al., 1994). Application of the revised version of the model to experimental data suggested that lung overload does not cause a dramatic increase in the total burden of the macrophage pool but results in a great increase in the particle burden of the interstitial space, a compartment that is not available for macrophage-mediated clearance. The revised version of the POCK model is discussed in greater detail in the context of other dosimetry models below.

Oberdörster and co-workers (1992) assessed the alveolar clearance of smaller (3.3 µm diam.) and larger (10.3 µm diam.) polystyrene particles, the latter of which are volumetrically equivalent to about 60% of the average normal volume of a rat AM, after intratracheal instillation into the lungs of rats. Even though both sizes of particles were found to be phagocytized by AMs within a day after deposition, and the smaller particles were cleared at a normal rate, only minimal lung clearance of the larger particles was observed over an approximately 200-day postinstillation period, thus supporting the volumetric overload hypothesis.

Animal studies have revealed that impairment of alveolar clearance can occur following chronic exposure to DPM (Griffis et al., 1983; Wolff et al., 1987; Vostal et al., 1982; Lee et al., 1983) or a variety of other diverse poorly soluble particles of low toxicity (Lee et al., 1986, 1988; Ferin and Feldstein, 1978; Muhle et al., 1990). Because high lung burdens of insoluble, biochemically-inert particles result in diminution of normal lung clearance kinetics or in what is now called particle overloading, this effect appears to be more related to the mass and/or volume of particles in the lung than to the nature of the particles per se. Particle overload only relates to poorly soluble articles of low toxicity. It must be noted, however, that some types of particles may be cytotoxic and impair clearance at lower lung burdens (e.g., silica may impair clearance at much lower lung burdens than DPM). Regardless, as pointed out by Morrow (1988), particle overloading in the lung modifies the dosimetry for particles in the lung and thereby can alter toxicologic responses.

Although quantitative data are limited regarding lung overload associated with impaired alveolar clearance in humans, impairment of clearance mechanisms appears to occur, and at a lung burden generally in the range reported to impair clearance in rats. Stöber et al. (1967), in their study of coal miners, reported lung particle burdens of 2 to 50 mg/g lung tissue, for which estimated clearance $t_{1/2}$ values were very long (4.9 years). Freedman and Robinson (1988) also reported slower alveolar clearance rates in coal miners, some of whom had a mild degree of pneumoconiosis. It must be noted, however, that no lung cancer was reported for those miners with apparent particle overload.

3.4.3. Potential Mechanisms for an AM Sequestration Compartment for Particles During Particle Overload

Several factors may be involved in the particle-load-dependent retardations in the rate of particle removal from the lung and the corresponding functional appearance of an abnormally slow clearing or particle sequestration compartment. As previously mentioned, one potential site for particle sequestration is the containment of particles in the Type I cells. Information on the retention kinetics for particles in the Type I cells is not currently available. Also, no morphometric analyses have been performed to date to estimate what fraction of a retained lung burden may be contained in the Type I cell population of the lung during lung overloading.

Another anatomical region in the lung that may be a slow clearing site is the interstitial compartment. Little is known about the kinetics of removal of free particles or particle-containing macrophages from the interstitial spaces, or what fraction of a retained burden of particles is contained in the lung's interstitium during particle overload. The gradual accumulation of particles in the regional lymph nodes and the appearance of particles and cells with associated particles in lymphatic channels and in the peribronchial and perivascular lymphoid tissue (Lee et al., 1985; White and Garg, 1981) suggest that the mobilization of particles from interstitial sites via local lymphatics is a continual process.

Indeed, it is clear from histologic observations of the lungs of animals chronically exposed to DPM that Type I cells, the interstitium, the lymphatic channels, and pulmonary lymphoid tissues could represent subcompartments of a more generalized slow clearing compartment.

Although these sites must be considered potential contributors to the increased retention of particles during particle overload, a disturbance in particle-associated AM-mediated clearance is undoubtedly the predominant cause, inasmuch as the AMs are the primary reservoirs of deposited particles. The factors responsible for a failure of AMs to translocate from the alveolar space compartment in lungs with high particulate matter burdens remain uncertain, although a hypothesis concerning the process has been offered involving volumetric AM burden (Morrow, 1988).

Other processes also may be involved in preventing particle-laden AMs from leaving the alveolar compartment under conditions of particle overload in the lung. Clusters or aggregates of particle-laden AMs in the alveoli are typically found in the lungs of laboratory animals that have received large lung burdens of a variety of types of particles (Lee et al., 1985), including DPM (White and Garg, 1981; McClellan et al., 1982). The aggregation of AMs may explain, in part, the reduced clearance of particle-laden AM during particle overload. The definitive mechanism(s) responsible for this clustering of AMs has not been elucidated to date. Whatever the underlying mechanism(s) for the AM aggregation response, it is noteworthy that AMs lavaged from the lungs of diesel exhaust-exposed animals continue to demonstrate a propensity to aggregate (Strom, 1984). This observation suggests that the surface characteristics of AMs are fundamentally

altered in a manner that promotes their adherence to one another in the alveolar region and that AM aggregation may not simply be directly caused by their abundant accumulation as a result of immobilization by large particle loads. Furthermore, even though overloaded macrophages may redistribute particle burden to other AMs, clearance may remain inhibited (Lehnert, 1988). This may, in part, be due to attractants from the overloaded AMs causing aggregation of those that are not carrying a particle burden.

3.5. MODELING THE DISPOSITION OF PARTICLES IN THE RESPIRATORY TRACT

3.5.1. Introduction

The biologic effects of inhaled particles are a function of their disposition. This, in turn, depends on their patterns of both deposition (i.e., the sites within which they initially come into contact with airway epithelial surfaces and the amount removed from the inhaled air at these sites) and clearance (i.e., the rates and routes by which deposited materials are removed from the respiratory tract). Removal of deposited materials involves the competing processes of macrophage-mediated clearance and dissolution-absorption. Over the years, mathematical models for predicting deposition, clearance and, ultimately, retention of particles in the respiratory tract have been developed. Such models help interpret experimental data and can be used to make predictions of deposition for cases where data are not available. A review of various mathematical deposition models was given by Morrow and Yu (1993) and in U.S. EPA (1996).

Currently available data for long-term inhalation exposures to insoluble particles (e.g., TiO_2, carbon black, and DPM) show that pulmonary retention and clearance of these particles are not adequately described by simple first-order kinetics and a single compartment representing the alveolar macrophage particle burden. Several investigators have developed models for deposition, transport, and clearance of insoluble particulate matter in the lungs. All of these models identify various compartments and associated transport rates, but empirically derived data are not available to validate many of the assumptions made in these models.

3.5.2. Dosimetry Models for DPM

3.5.2.1. *Introduction*

Diesel particles are irregularly shaped aggregates with a mass median aerodynamic diameter (MMAD) of approximately 0.2 μm, formed from primary spheres 15-30 nm in diameter. The primary sphere consists of a dense carbonaceous core (soot) on which various combustion-derived organic compounds, accounting for 10% to 30% of the particle mass, are adsorbed.

The extrapolation of toxicological results from laboratory animals to humans requires the use of dosimetry models for both species that include, first, the deposition of DPMs in various regions of the respiratory tract, and second, the transport and clearance of the particles from their

deposited sites. Particles deposit by impaction, sedimentation, interception, and diffusion. The contribution from each mechanism is a function of particle size, lung structure, and size and breathing parameters. Because of the size of diesel particles, under normal breathing conditions most of this deposition takes place by diffusion, and the fraction of the inhaled mass that is deposited in the thoracic region is substantially similar for rats and humans. The clearance of particles takes place (1) by mechanical processes: mucociliary transport in the ciliated conducting airways and macrophage phagocytosis and migration in the nonciliated airways, and (2) by dissolution. The removal of the carbonaceous soot is largely by mechanical clearance, whereas the clearance of the adsorbed organics is principally by dissolution.

3.5.2.2. *Deposition Models*

Among deposition models that include aspects of lung structure and breathing dynamics, the most widely used have been typical-path or single-path models (Yu, 1978; Yu and Diu, 1983). The single-path models are based on an idealized symmetric geometry of the lung, assuming regular dichotomous branching of the airways and alveolar ducts (Weibel, 1963). They lead to modeling the deposition in an average regional sense for a given lung depth. Although the lower airways of the lung may be reasonably characterized by such a symmetric representation, there are major asymmetries in the upper airways of the tracheobronchial tree that in turn lead to different apportionment of airflow and particulate burden to the different lung lobes. The rat lung structure is highly asymmetric because of its monopodial nature, leading to significant errors in a single-path description. This is rectified in the multiple-path model of the lung that incorporates asymmetry and heterogeneity in lung branching structure, and calculates deposition at the individual airway level. This model has been developed for the rat lung by Anjilvel and Asgharian (1995) and, in a limited fashion because of insufficient morphometric data, for the human lung (Subramaniam et al., 1998; Yeh and Schum, 1980). Such models are particularly relevant for fine and ultrafine particles. However, models for clearance have not yet been implemented in conjunction with the use of the multiple-path model. Therefore, in this report we use only the single-path model in deposition calculations, specifically the works by Yu and Xu (1986) and Xu and Yu (1987).

3.5.2.3. *Physiologically Based Models for Clearance*

Several clearance models currently exist, and these differ significantly in the level of physiological detail that is captured in the model and in the uncertainties associated with the values of the parameters used. All of these models identify various compartments and associated transport rates, but empirically derived data are not available to validate many of the assumptions made in the models. We compare four of the most widely discussed models below.

3.5.2.3.1. ***Two-compartment model.*** Currently available data for long-term inhalation exposures to insoluble particles (e.g., TiO_2, carbon black, and DPM) show that pulmonary retention and clearance of these particles are not adequately described by simple first-order kinetics and a single compartment representing the alveolar macrophage particle burden. A two-compartment model was developed by Smith (1985) that includes alveolar and interstitial compartments. For uptake and clearance of particles by alveolar surface macrophages and interstitial encapsulation of particles (i.e., quartz dust), available experimental data show that the rate-controlling functions followed Michaelis-Menton type kinetics, while other processes affecting particle transfer are assumed to be linear. Although this model provides rate constants as functions that vary depending on the conditions within the various compartments, most of the described functions could not be validated with experimental data.

3.5.2.3.2. ***Multicompartmental models.*** Strom et al. (1988) developed a multicompartmental model for particle retention that partitioned the alveolar region into two compartments on the basis of the physiology of clearance. The alveolar region has a separate compartment for sequestered macrophages, which corresponds to phagocytic macrophages that are heavily laden with particles and clustered, and therefore have significantly lowered mobility. The model has the following compartments: (1) tracheobronchial tree, (2) free particulate on the alveolar surface, (3) mobile phagocytic alveolar macrophages, (4) sequestered particle-laden alveolar macrophages, (5) regional lymph nodes, and (6) gastrointestinal tract. The model is based on mass-dependent clearance (the rate coefficients reflect this relationship), which dictates sequestration of particles and their eventual transfer to the lymph nodes. The transport rates between various compartments were obtained by fitting the calculated results to lung and lymph node burden experimental data for both exposure and postexposure periods. Since the number of fitted parameters was large, the model is not likely to provide unique solutions that would simulate experimental data from various sources and for different exposure scenarios. For the same reason, it is not readily possible to use this model for extrapolating to humans.

3.5.2.3.3. ***POCK model.*** Stöber and co-workers have worked extensively in developing models for estimating retention and clearance of biologically insoluble, respirable particles in the lung. Their most recent work (1994), a revised version of the POCK (physiologically oriented multicompartmental kinetic) model, is a rigorous attempt to incorporate most of the physiologically known aspects of alveolar clearance and retention of inhaled insoluble particles. Their multicompartmental kinetics model has five subcompartments. The transfer of particles between any of the compartments within the alveolar region is macrophage-mediated. There are two compartments that receive particles cleared from the alveolar regions: the tracheobronchial tract and the lymphatic system.

The macrophage pool includes both mobile and particle-laden, immobilized macrophages. The model assumes a constant maximum volume capacity of the macrophages for particle uptake and a material-dependent critical macrophage load that results in total loss of macrophage mobility. Sequestration of those macrophages heavily loaded with a particle burden close to a volume load capacity is treated in a sophisticated manner by approximating the particle load distribution in the macrophages. The macrophage pool is compartmentalized in terms of numbers of macrophages that are subject to discrete particle load intervals. Upon macrophage death, the phagocytized particle is released back to the alveolar surface; thus phagocytic particle collection competes to some extent with this release back to the alveolar surface. This recycled particle load is also divided into particle clusters of size intervals defining a cluster size distribution on the alveolar surface. The model yields a time-dependent frequency distribution of loaded macrophages that is sensitive to both exposure and recovery periods in inhalation studies.

The POCK model also emphasizes the importance of interstitial burden in the particle overload phenomenon and indicates that particle overload is a function of a massive increase in particle burden of the interstitial space rather than total burden of the macrophage pool. The relevance of the increased particle burden in the interstitial space lies with the fact that this compartmental burden is not available for macrophage-mediated clearance and, therefore, persists even after cessation of exposure.

While the POCK model is the most sophisticated in the physiological complexity it introduces, it suffers from a major disadvantage. Experimental retention studies provide data on total alveolar and lymph node mass burdens of the particles as a function of time. The relative fraction of the deposit between the alveolar subcompartments in the Stöber model therefore cannot be obtained experimentally; the model thus uses a large number of parameters that are simultaneously fit to experimental data. Although the model predictions are tenable, experimental data are not currently available to validate the proposed compartmental burdens or the transfer rates associated with these compartments. Thus the over-parameterization in the model leads to the problem that the model may not provide a unique solution that may be used for a variety of exposure scenarios, and for the same reason, cannot be used for extrapolation to humans. Stöber et al. have not developed an equivalent model for humans; therefore the use of their model in our risk assessment for diesel is not attempted.

3.5.2.3.4. ***Yu-Yoon model.*** Yu and Yoon (1990), on the other hand, have developed a three-compartment lung model that consists of tracheobronchial (T), alveolar (A), and lymph node (L) compartments (Appendix B, Figure B-1) and, in addition, considered filtration by a nasopharyngeal or head (H) compartment. Absorption by the blood (B) and gastrointestinal (G) compartments was also considered. While the treatment of alveolar clearance is physiologically less sophisticated than that of the Stöber et al. model, the Yu-Yoon model provides a more

comprehensive treatment of clearance by including systemic compartments and the head, and including the clearance of the organic components of DPM in addition to the insoluble carbon core.

The tracheobronchial compartment is important for short-term considerations, while long-term clearance takes place via the alveolar compartment. In contrast to the Stöber and Strom approaches, the macrophage compartment in the Yu-Yoon model contains all of the phagocytized particles; that is, there is no separate (and hypothetical) sequestered macrophage subcompartment. Instead, in order to progress beyond the classical retention model (International Commission on Radiological Protection, 1979), Yu and Yoon have addressed the impairment of long-term clearance (the overload effect) by using a set of variable transport rates for clearance from the alveolar region as a function of the mass of DPM in the alveolar compartment. A functional relationship for this was derived mathematically (Yu et al., 1989) based upon Morrow's hypothesis for the overload effect that we discussed earlier in the section on pulmonary overload. The extent of the impairment depends on the initial particle burden, with greater particulate concentration leading to slower clearance.

DPM are treated as composed of three material components: an insoluble carbonaceous core, slowly cleared organics (10% particle mass), and fast-cleared organics (10% particle mass). Such a partitioning of organics was based on observations that the retention of particle-associated organics in lungs shows a biphasic decay curve (Sun et al., 1984; Bond et al., 1986). For any compartment, each of these components has a different transport rate. The total alveolar clearance rate of each material component is the sum of clearance rates of that material from the alveolar to the tracheobronchial, lymph, and blood compartments. In the Strom and Stöber models discussed above, the clearance kinetics of DPM were assumed to be entirely dictated by that of the insoluble carbon core. For those organic compounds that get dissociated from the carbon core, clearance rates are likely to be very different, and some of these compounds may be metabolized in the pulmonary tissue or be absorbed by blood.

The transport rates were derived from experimental data for rats using several approximations. The transport rates for the carbonaceous core and the organic components were derived by fitting to data from separate experiments. Lung and lymph node burdens from the experiment of Strom et al. (1988) were used to determine the transport rate of the carbon core. The Yu-Yoon model incorporates the impairment of clearance by including a mass dependency in the transport rate. This mass dependency is easily extracted because the animals in the experiment were killed over varying periods following the end of exposure.

It was assumed that the transport rates from the alveolar and lymph compartments to the blood were equal and independent of the particulate mass in the alveolar region. The clearance rates of particle-associated organics for rats were derived from the retention data of Sun et al.

(1984) for benzo[a]pyrene and the data of Bond et al. (1986) for nitropyrene adsorbed on diesel particles.

3.5.2.4. *Model Assumptions and Extrapolation to Humans*

The Yu-Yoon approach takes the perspective that parsimonious models are to be preferred in order to enable experimental validation and extrapolation from rats to humans.

Yu and Yoon make two important assumptions to carry out the extrapolation in the light of inadequate human data. First, the transport rates of organics in the DPM do not change across species. This is based upon lung clearance data of inhaled lipophilic compounds (Schanker et al., 1986), where the clearance was seen to be dependent on the lipid/water partition coefficient. In contrast, the transport rate of the carbon core is considered to be significantly species-dependent (Bailey et al., 1982). DPM clearance rate is determined by two terms in the model (see equation C-82). The first, corresponding to macrophage-mediated clearance, is a function of the lung burden, and is assumed to vary significantly across species. The second term, a constant, corresponding to clearance by dissolution, is assumed to be species-independent. The mass-dependent term for humans is assumed to vary in the same proportion as in rats under the same unit surface particulate dose. The extrapolation is then achieved by using the data of Bailey et al. (1982) for the low lung burden limit of the clearance rate. This value of 0.0017/day was lower than the rat value by a factor of 7.6. This is elaborated further in Appendix C. Other transport rates that have lung burden dependence are extrapolated in the same manner.

The Bailey et al. experiment, however, used fused monodisperse aluminosilicate particles of 1.9 and 6.1 μm aerodynamic diameters. Yu and Yoon have used the longer of the half-times obtained in this experiment; in using such data for diesel soot particles 0.2 μm in diameter, they have assumed the clearance of insoluble particles to be independent of size over this range. This appears to be a reasonable assumption since the linear dimensions of an alveolar macrophage is significantly larger, roughly 10 μm (Yu et al., 1996). However, Snipes (1979) has reported a clearance rate (we convert here from their half-life values) of 0.0022/day for 1 and 2 μm particles but a higher value of 0.0039/day for 0.4 μm particles. In the absence of reliable data for 0.2 μm particles, clearance rate pertaining to a much larger particle size is being used. Although such a choice may underestimate the correct clearance rate for DPM, the resulting error in the human equivalent concentration is likely to be only more protective of human health. Long-term clearance rates for particle sizes more comparable to DPM are available, e.g., iron oxide and polystyrene spheres (Waite and Ramsden, 1971; Jammet et al., 1978), but these data show a large range in the values obtained for half-lives or are based upon a very small number of trials, and therefore compare unfavorably with the quality of data from the Bailey experiment.

The deposition fractions of particulate matter in the pulmonary and tracheobronchial regions of the human lung remain relatively unchanged over the particle size range between

0.2 and 1.0 µm. Since the clearance of insoluble particles is also likely to remain the same over this range, the dosimetry results in this report for the carbon core component of DPM could also be extended to other particles in this size range within the PM2.5. For particle diameters between 1.0 and 3.5 µm, the deposition fraction in the pulmonary region increases significantly (Yu and Diu, 1983), so the diesel model will not be applicable for particles in this range without changing the value for the deposition fractions.

Although there was good agreement between experiment and calculated results, this agreement follows a circular logic (as adequately pointed out by Yu and Yoon [1990]) because the same experimental data figured into the derivation of transport rates used in the model. Nevertheless, while this agreement is not a validation, it provides an important consistency check on the model. Thus, the model awaits further experimental data for a reasonable validation.

The model showed that at low lung burdens, alveolar clearance is dominated by mucociliary transport to the tracheobronchial region, and at high lung burdens, clearance is dominated by transport to the lymphatic system. The head and tracheobronchial compartments showed quick clearance of DPM by mucociliary transport and dissolution. Lung burdens of both the carbon core and organics were found to be greater in humans than in rats for similar periods of exposure.

The Yu-Yoon publication provides a parametric study of the dosimetry model, examining variation over a range of exposure concentrations, breathing scenarios and ventilation parameters, particle mass median aerodynamic diameters, and geometric standard deviations of the aerosol distribution. It examines how lung burden varies with age for exposure over a life span, provides dosimetry extrapolations to children, and examines changes in lung burden with lung volume. The results showed that children would exhibit more diminished alveolar clearance of DPM at high lung burden than adults when exposed to the equal concentrations of DPM. These features make the model easy to use in risk assessment studies. We refer the reader to Appendix C for further details on the model and for analyses of the sensitivity of the model to change in parameter values.

The Yu-Yoon model presents some uncertainties in addition to those discussed earlier in the context of particle size dependence of clearance rate. The Yu and Yoon report underwent extensive peer review; we list below the most important among the model uncertainties discussed by the review panel. The experimental data used by the Yu-Yoon model for adsorbed organics used passively adsorbed radiolabeled compounds as surrogates for combustion-derived organics. These compounds may adhere differently to the carbon core than those formed during combustion. Yu and Yoon have estimated that slowly cleared organics represent 10% of the total particle mass; the actual figure could be substantially less; the reviewers estimate that the amount of tightly bound organics is probably only 0.1% to 0.25% of the particle mass.

The model was based upon the experimental data of Strom et al. (1988) where Fischer-344 rats were exposed to DPM at a concentration of 6.0 mg/m^3 for 20 hours/day and 7

days/week for periods ranging from 3 to 84 days. Such exposures lead to particle overload effects in rats, whereas human exposure patterns are usually of much lower levels at which overload will not occur. Secondly, human exposures are not likely to be continuous, but most likely over brief periods of time. Parameters obtained by fitting to data under the conditions of the experimental scenario for rats may not be optimal for the human exposure and concentration of interest.

The extrapolation of retained dose from rats to humans assumed that the macrophage-mediated mechanical clearance of the DPM varies with the specific particulate dose to the alveolar surface in the same proportion in humans and in rats, whereas clearance rates by dissolution were assumed to be invariant across species. This assumption has not been validated.

3.5.3. Deposition of Organics

Using the data presented by Xu and Yu (1987), it is possible to calculate the total mass of DPM, as well as the total organic mass and specific carcinogenic PAHs deposited in the lungs of an individual exposed to DPM. For example, the annual deposition of DPM in the lungs of an individual exposed continuously to 1 $\mu g/m^3$ DPM can be estimated to be about 420 µg. About 0.7% of particle mass consists of PAHs (see Section 2.2.6.2, Chapter 2) for a total of 2.94 µg. Of this amount, the deposited mass of nitro-polycyclic aromatic compounds based on data by Campbell and Lee (1984) would equal 37 ng, while the deposited mass of 7 PAHs that tested positive in cancer bioassays (U.S. EPA, 1993), and measured by Tong and Karasek (1984), would range from 0.16 to 0.35 µg. While these amounts are very small, exposure concentrations are often greater than 1 $\mu g/m^3$, and deposition in humans can be expected to be concentrated at limited sites, especially at the bifurcations of the small bronchi.

3.6. BIOAVAILABILITY OF ORGANIC CONSTITUENTS PRESENT ON DIESEL EXHAUST PARTICLES

Because it has been shown that DPM extract is not only mutagenic but also contains known carcinogens, the organic fraction was originally considered to be the primary source of carcinogenicity in animal studies. Since then evidence has been presented that carbon black, lacking an organic component, is capable of inducing lung cancer at exposure concentrations sufficient to induce lung particle overload. This suggested that the insoluble carbon core of the particle may be of greater importance for the pathogenic and carcinogenic processes observed in the rat inhalation studies conducted at high exposure concentrations. (See Chapter 7 for a discussion of this issue.) Nevertheless, because lung tumor induction was reported in epidemiology studies at exposure levels unlikely to induce lung particle overload, it is reasonable to assume that organic compounds play a role.

The bioavailability of toxic organic compounds adsorbed to particles can be influenced by a variety of factors. Although the agent may be active while present on the particle, most

particles are taken up by AMs, a cell type not generally considered to be a target site. In order to reach the target site, elution from the particle surface is necessary followed by diffusion and uptake by the target cell. Metabolism to an active form by either the phagocytes or the target cells is also required for activity of many of the compounds present.

3.6.1. In Vivo Studies

3.6.1.1. *Laboratory Investigations*

Several studies reported on the retention of particle-adsorbed organics following administration to various rodent species. In studies reported by Sun et al. (1982, 1984) and Bond et al. (1986), labeled organics were deposited on diesel particles following heating to vaporize the organics originally present. Sun et al. (1982) compared the disposition of either pure or diesel particle-adsorbed benzo[a]pyrene B[a]P following nose-only inhalation by F344 rats. About 50% of particle-adsorbed B[a]P was cleared with a half-time of 1h predominantly by mucociliary clearance. The long-term retention of particle-adsorbed ^{3}H-B[a]P (18 days) was approximately 230-fold greater than that for pure ^{3}H-B[a]P (Sun et al., 1982). At the end of exposure, about 15% of the ^{3}H label was found in blood, liver, and kidney. Similar results were reported in a companion study by Bond et al. (1986), and by Sun et al. (1984) with another PAH, 1-nitropyrene, except retention half-time was 36 days.

Ball and King (1985) studied the disposition and metabolism of intratracheally instilled ^{14}C-labeled 1-NP (>99.9% purity) coated onto DPM. About 50% of the ^{14}C was excreted within the first 24 h; 20% to 30% of this appeared in the urine, and 40% to 60% was excreted in the feces. Traces of radiolabel were detected in the trachea and esophagus. Five percent to 12% of the radiolabel in the lung co-purified with the protein fraction, indicating protein binding of the 1-NP-derived ^{14}C. However, the corresponding DNA fraction contained no ^{14}C above background levels.

Bevan and Ruggio (1991) assessed the bioavailability of B[a]P adsorbed to DPM from a 5.7-L Oldsmobile engine. In this study, exhaust particles containing 1.03 µg B[a]P/g particles were supplemented with exogenous ^{3}H-B[a]P to provide 2.62 µg B[a]P/g of exhaust particles. In vitro analysis indicated that the supplemented B[a]P eluted from the particles at the same rate as the original B[a]P. Twenty-four hours after intratracheal instillation in Sprague-Dawley rats, 68.5% of the radiolabel remained in the lungs. This is approximately a 3.5-fold greater proportion than that reported by Sun et al. (1984), possibly because smaller amounts of B[*a*]P adsorbed on the particles, resulting in stronger binding. At 3 days following administration, over 50% of the radioactivity remained in the lungs, nearly 30% had been excreted into the feces, and the remainder was distributed throughout the body. Experiments using rats with cannulated bile ducts showed that approximately 10% of the administered radioactivity appeared in the bile over a 10-h period and that less than 5% of the radioactivity entered the feces via mucociliary transport.

Results of these studies showed that the retention of organics in the lungs is increased considerably when organics are adsorbed to diesel particles. Because retention time is very short following exposure to the pure compounds, it can be concluded that the increased retention time is primarily the result of continued binding to the particles. The detection of labeled compounds in blood, distant organs, urine, and bile as well as the trachea, however, provides evidence that at least some of the organics are eluted from the particles following deposition in the lungs.

3.6.1.2. *Studies in Occupationally Exposed Humans*

DNA adduct induction in the lungs of experimental animals exposed to diesel exhaust have been measured in a number of animal experiments (see World Health Organization [1996] for a review). Such studies, however, provide limited information regarding bioavailability of organics, since positive results may well have been related to factors associated with lung particle overload. In fact, Bond et al. (1990) reported that carbon black, which is virtually devoid of organics, is capable of inducing DNA adducts in rats at lung overload doses.

On the other hand, DNA adduct formation and/or mutations in blood cells following exposure to DPM, especially at levels insufficient to induce lung overload, can be presumed to be the result of organics diffusing into the blood. Hemminki et al. (1994) reported increased levels of DNA adducts in lymphocytes of bus maintenance and truck terminal workers. Österholm et al. (1995) studied mutations at the hprt-locus of T-lymphocytes in bus maintenance workers. Although they were unable to identify clearcut exposure-related differences in types of mutations, adduct formation was significantly increased in the exposed workers. Nielsen et al. (1996) reported significantly increased levels of lymphocyte DNA adducts, hydroxyvaline adducts in hemoglobin, and 1-hydroxypyrene in urine of garage workers exposed to diesel exhaust.

3.6.2. In Vitro Studies

3.6.2.1. *Extraction of Diesel Particle-Associated Organics by Biological Fluids*

In vitro extraction of organics in biological fluids can be estimated by measurement of mutagenic activity. Using this approach, Brooks et al. (1981) reported extraction efficiencies of only 3% to 10 % that of dichloromethane following DPM incubation in lavage fluid, serum, saline, albumin, dipalmitoyl lecithin, or dichloromethane. Moreover, extraction efficiency did not increase with incubation time up to 120 h. Similar findings were reported by King et al. (1981). In the latter study, lung lavage fluid and lung cytosol fluid extracts of DPM were not mutagenic. Serum extracts of DPM did exhibit some mutagenic activity, but considerably less than that for organic solvent extracts. Furthermore, the mutagenic activity of the solvent extract was significantly reduced when combined with serum or lung cytosol fluid, suggesting protein binding or biotransformation of the mutagenic components. Siak et al. (1980) assessed the mutagenicity of material extracted from DPM by bovine serum albumin in solution, simulated lung surfactant,

fetal calf serum (FCS), and physiologic saline. Only FCS was found to extract some mutagenic activity from the DPM.

Despite the apparent inability of biological fluids to extract organics in vitro, their effectiveness in vivo remains equivocal because of differing conditions. Extracellular lung fluid is a complex mixture of constituents that undoubtedly have a broad range of hydrophobicity (George and Hook, 1984; Wright and Clements, 1987), and it fundamentally differs from serum in terms of chemical composition (Gurley et al., 1988). Moreover, assessments of the ability of lavage fluids, which actually represent substantially diluted extracellular lung fluid, to extract mutagenic activity from DPM clearly do not reflect the in vivo condition. Finally, except under very high exposure concentrations, few particles escape phagocytosis and possible intracellular extraction.

3.6.2.2. *Extraction of Diesel Particle-Associated Organics by Lung Cells and Cellular Components*

A more likely means by which organic carcinogens (e.g., PAHs) may be extracted from DPM and metabolized in the lung is either particle dissolution or extraction of organics from the particle surface within the phagolysosomes of AMs and other lung cells. This mechanism presupposes that the particles are internalized. Specific details about the physicochemical conditions of the intraphagolysosomal environment, where particle dissolution in AMs presumably occurs in vivo, have not been well characterized. However, it is known that the phagolysosomes constitute an acidic (pH 4 to 5) compartment in macrophages (Nilsen et al., 1988; Ohkuma and Poole, 1978). The relatively low pH in the phagolysosomes has been associated with the dissolution of some types of inorganic particles (some metals) by macrophages (Marafante et al., 1987; Lundborg et al., 1984), but few studies provide quantitative information concerning how organic constituents of DPM (e.g., B[a]P) may be extracted in the phagolysosomes (Bond et al., 1983). Whatever the mechanism, assuming elution occurs, the end result is a prolonged exposure of the respiratory epithelium to low concentrations of carcinogenic agents.

Early studies by King et al. (1981) found that when pulmonary alveolar macrophages were incubated with DPM, amounts of organic compounds and mutagenic activity decreased measurably from the amount originally associated with the particles, suggesting that organics were removed from the phagocytized particles. Leung et al. (1988) studied the ability of rat lung and liver microsomes to facilitate transfer and metabolism of B[*a*]P from diesel particles. ^{14}C-B[*a*]P coated diesel particles, previously extracted to remove the original organics present, were incubated with liver or lung microsomes. About 3% of the particle adsorbed B[*a*]P was transferred to the lung microsomes within 2 h. Of this amount about 1.5% was metabolized, for a total of about 0.05% of the B[*a*]P adsorbed to the DPM. While transformation is slow, because

of long retention half-lives of particles in humans the fraction eluted and metabolized may well be significant.

In analyzing phagolysosomal dissolution of various ions from particles in the lungs of Syrian golden hamsters, however, Godleski et al. (1988) demonstrated that solubilization did not necessarily result in clearance of the ions and that binding of the solubilized components to cellular and extracellular structures occurred. It is reasonable to assume that phagocytized DPM particles may be subject to similar processes and that these processes would be important in determining the rate of bioavailability of the particle-bound constituents of DPM.

3.6.3. Modeling Studies

Gerde et al. (1991a,b) described a model simulating the effect of particle aggregation and PAH content on the rate of PAH release in the lung. According to this model, particle aggregation will occur with high exposure concentrations, resulting in a slow release of PAHs with prolonged exposure to surrounding tissues. However, large aggregates of inert dust are unlikely to form at doses typical of human exposures. Inhaled particles, at low concentrations, are more likely to deposit and react with surrounding lung medium without interference from other particles. The model predicts that, under low-dose exposure conditions more typical in humans, particle-associated organics will be released more rapidly from the particles because they are not aggregated. Sustained exposure of target tissues to PAHs will result from repeated exposures, not from increased retention due to association of PAHs with carrier particles. This distinction is important because at low doses PAH exposure and lung tumor formation should occur at sites of deposition rather than retention as occurs with high doses.

The site of release of PAHs influences effective dose to the lungs because, as noted previously, at least some free organic compounds such as B[*a*]P deposited in the lungs are rapidly absorbed into the bloodstream. Gerde et al. (1991b) predicted lipophilic PAHs would be retained in the alveoli less than 1 min, whereas they may be retained for hours in the bronchi. These predictions were based on an average diffusion distance to capillaries of only about 0.5 µm in the alveoli, whereas in the bronchi it probably exceeds 50 µm. An experimental study by Gerde et al. (1999) provided support for this prediction. Beagle dogs were exposed to ^{3}H-B[*a*]P adsorbed on the carbonaceous core of diesel particles at a concentration of 15 µg B[*a*]P/gm particles. A rapidly eluting fraction from particles deposited in the alveoli was adsorbed into the bloodstream and metabolized in the liver. The rapidly eluting fraction from particles deposited in the conducting airways, however, was to a large extent retained and metabolized in airway epithelium.

Nikula et al. (1997) reported that 52% of DPM deposited in the lungs of Cynomolgus monkeys chronically inhaling diesel exhaust were found in the interstitium of small airways, compared with 27% in rats (Nikula et al., 1997). This is primarily due to a lack of respiratory

bronchioles in the rat. Because lung structure is similar in monkeys and humans, a significantly greater percentage of DPM matter can be predicted to deposit in small airway branches of humans. Overall, bioavailability of organics in humans is expected to be greatest at bifurcations of small airways because they are a major site of particle deposition, have a longer residence time for eluted organics in airways than alveoli, allow time for uptake and metabolism by airway epithelium, and predict more rapid elution from particles at ambient exposure concentrations.

Overall, the results of studies presented in Section 3.6 provide evidence that at least some of the organic matter adsorbed to DPM deposited in the lungs is eluted. However, the percentage taken up and metabolized to an active form by target cells is uncertain. Organics eluted from particles deposited in alveoli are likely to rapidly enter the bloodstream and pose little risk for induction of lung pathology and/or cancer. Risk of harmful effects for particles deposited in the small airways is predicted to be greater because solubilized organic compounds will be retained longer, allowing for metabolism by epithelial cells lining the airways. Since the deposition in small airways occurs primarily at bifurcations, localized higher concentrations will occur. At present, unfortunately, the available data are insufficient to accurately model the effective dose of organics in the respiratory tract of humans or animals exposed to diesel exhaust, especially at specific target sites such as small airway branchings.

3.7. SUMMARY

The most consistent historical measure of dose for diesel emissions is DPM in units of µg particles/m^3. With the assumption that all components of diesel emissions (e.g., organics in the form of volatilized liquids or gases) are present in proportion to the DPM mass, DPM can be used as the basic dosimeter for effects from various scenarios including acute and chronic exposures, as well as for different endpoints such as irritation, fibrosis, or even cancer. There is, however, little evidence currently available to prove or refute DPM as being the most appropriate dosimeter.

The DPM dose to the tissue is related to the extent of the deposition and clearance of DPM. Diesel exhaust particles may deposit throughout the respiratory tract via sedimentation or diffusion, with the latter being prevalent in the alveolar region. Particles that deposit upon airway surfaces may be cleared from the respiratory tract completely or may be translocated to other sites by regionally distinct processes that can be categorized as either absorptive (i.e., dissolution) or nonabsorptive (i.e., transport of intact particles via mucociliary transport). With insoluble or poorly soluble particles such as DPM, clearance by dissolution is insignificant compared to the rate of clearance as an intact particle. Another mechanism that can affect retention of DPM is endocytosis by alveolar lining cells that, in turn, can lead to the accumulation of DPM in the interstitial compartment of the lung and subsequent translocation of DPM to lymph nodes. For poorly soluble particles such as DPM, species-dependent rate constants exist for the various clearance pathways that can be modified by factors such as respiratory tract disease.

In rats, prolonged exposure to high particle concentrations may be associated with what is termed particle overload. This condition is defined as the overwhelming of macrophage-mediated clearance by the deposition of particles at a rate exceeding the capacity of that clearance pathway, occurring in rats when deposition approaches 1 mg particles/g lung tissue. The relevance of lung overload to humans, and even to species other than laboratory rats and mice, is problematic. Relevance to humans is further clouded by the suggestion that macrophage-mediated clearance is normally slower and perhaps less important in humans than in rats. Whereas such clearance is likely to be of little relevance for most "real-world" ambient exposures of humans, it is of concern in interpreting some long-term experimental exposure data and perhaps for some human occupational exposures.

Extrapolation of toxicological results from laboratory animals to humans to obtain an HEC requires the use of a dosimetry model incorporating critical aspects of both species that include (1) the deposition of DPM in various regions of the respiratory tract, and (2) the transport and clearance of the particles from their deposited sites. Review and evaluation of the models available led to the choice of the Yu and Yoon (1990) model. This model has a three-compartment lung consisting of tracheobronchial, alveolar, and lymph node compartments and, in addition, considers filtration by a nasopharyngeal or head compartment. Absorption by the blood and gastrointestinal compartments was also considered. In addition, the model treats DPM as being composed of the insoluble carbonaceous core, slowly cleared organics, and fast-cleared organics. Major assumptions made in this model include that transport rates of organics in DPM do not change across species and that the transport rate of the carbonaceous core is species dependent such that the clearance varies with specific dose to the alveolar surface in the same proportion in humans and in rats. This model was used to project HECs from concentrations used in experimental animal exposures. Use of HECs partially obviates the need for an animal-to-human uncertainty factor, as explained in Chapter 9.

The degree of bioavailability of the organic fraction of DPM is still somewhat uncertain. However, reports of DNA induction in occupationally exposed workers as well as results of animal studies using radiolabeled organics deposited on diesel particles indicate that at least a fraction of the organics present are eluted prior to particle clearance. In addition, data have been presented indicating that a greater percentage of diesel particles are deposited in the branching of small airways of laboratory primates, and presumably of humans, than in those of rats. Carcinogenic organics eluted in this region remain in the lung long enough to be metabolized to an active form. Some of the toxicologically significant compounds, however, do not require metabolic activation. While adequate quantitative data are lacking, they do suggest the likely involvement of particle-associated organics in the carcinogenic process.

3.8. REFERENCES

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4. MUTAGENICITY OF DIESEL EXHAUST

Since 1978, more than 100 publications have appeared in which genotoxicity assays were used with diesel emissions, volatile and particulate fractions (including extracts), or individual chemicals found in diesel emissions. Although most of the studies deal with whether particulate extracts from diesel emissions possess mutagenic activity in microbial and mammalian cell assays, a number of studies in recent years have employed bioassays (most commonly Salmonella TA98 without S9) to evaluate (1) extraction procedures, (2) fuel modifications, (3) bioavailability of chemicals from diesel particulate matter (DPM), and (4) exhaust filters or other modifications and other variables associated with diesel emissions. As indicated in Chapter 2, the number of chemicals in diesel emissions is very large. Many of these (e.g. PAHs and nitro-PAHs) have been determined to exhibit mutagenic activity in a variety of assay systems. Because of the limited and uncertain role of the individual chemicals in either the cancer or noncancer effects of diesel emissions, discussion of those data are not included. Also, several review articles, some containing more detailed descriptions of the available studies, are available (International Agency for Research on Cancer, 1989) (Claxton, 1983; Pepelko and Peirano, 1983; Shirnamé-Moré, 1995). The proceedings of several symposia on the health effects of diesel emissions (U.S. EPA, 1980; Lewtas, 1982; Ishinishi et al., 1986) are also available. An understanding of diesel exhaust mutagenicity is important to the cancer health effects and dose-response chapters, Chapters 7 and 8, respectively.

4.1. GENE MUTATIONS

Huisingh et al. (1978) demonstrated that dichloromethane extracts from DPM were mutagenic in strains TA1537, TA1538, TA98, and TA100 of *S. typhimurium*, both with and without rat liver S9 activation. This report contained data from several fractions as well as DPM from different vehicles and fuels. Similar results with diesel extracts from various engines and fuels have been reported by a number of investigators using the Salmonella frameshift-sensitive strains TA1537, TA1538, and TA98 (Siak et al., 1981; Claxton, 1981; Dukovich et al., 1981; Brooks et al., 1984). Similarly, mutagenic activity was observed in Salmonella forward mutation assays measuring 8-azaguanine resistance (Claxton and Kohan, 1981) and in *E. coli* mutation assays (Lewtas, 1983).

One approach to identifying significant mutagens in chemically complex environmental samples such as diesel exhaust or ambient particulate extracts is the combination of short-term bioassays with chemical fractionation (Scheutzle and Lewtas, 1986). The analysis is most frequently carried out by sequential extraction with increasingly polar or binary solvents. Prefractionation by silica-column chromatography separates compounds by polarity or into acidic,

basic, and neutral fractions. The resulting fractions are too complex to characterize by chemical methods, but the bioassay analysis can be used to determine fractions for further analysis. In most applications of this concept, Salmonella strain TA98 without the addition of S9 has been used as the indicator for mutagenic activity. Generally, a variety of nitrated polynuclear aromatic compounds have been found that account for a substantial portion of the mutagenicity (Liberti et al., 1984; Schuetzle and Frazer, 1986; Schuetzle and Perez, 1983). However, not all bacterial mutagenicity has been identified in this way, and the identity of the remainder of the mutagenic compounds remains unknown. The nitrated aromatics thus far identified in diesel exhaust were the subject of review in the IARC monograph on diesel exhaust (International Agency for Research on Cancer, 1989). In addition to the simple qualitative identification of mutagenic chemicals, several investigators have used numerical data to express mutagenic activity as activity per distance driven or mass of fuel consumed. These types of calculations have been the basis for estimates that the nitroarenes (both mono- and dinitropyrenes) contribute a significant amount of the total mutagenic activity of the whole extract (Nishioka et al., 1982; Salmeen et al., 1982; Nakagawa et al., 1983). In a 1983 review, Claxton discussed a number of factors that affected the mutagenic response in Salmonella assays. Citing the data from the Huisingh et al. (1978) study, the author noted that the mutagenic response could vary by a factor of 100 using different fuels in a single diesel engine. More recently, Crebelli et al. (1995) used Salmonella to examine the effects of different fuel components. They reported that while mutagenicity was highly dependent on aromatic content, especially di- or triaromatics, there was no clear effect of sulfur content. Later, Sjögren et al. (1996), using multivariate statistical methods with 10 diesel fuels, concluded that the most influential chemical factors in Salmonella mutagenicity were sulfur contents, certain polycyclic aromatic hydrocarbons (PAHs) (1-nitropyrene), and naphthenes.

Matsushita et al. (1986) tested particle-free diesel exhaust gas and a number of benzene nitro-derivatives and PAHs (many of which have been identified as components of diesel exhaust gas). The particle-free exhaust gas was positive in both TA100 and TA98, but only without S9 activation. Of the 94 nitrobenzene derivatives tested, 61 were mutagenic, and the majority showed greatest activity in TA100 without S9. Twenty-eight of 50 PAHs tested were mutagenic, all required the addition of S9 for detection, and most appeared to show a stronger response in TA100. When 1,6-dinitropyrene was mixed with various PAHs or an extract of heavy-duty (HD) diesel exhaust, the mutagenic activity in TA98 was greatly reduced when S9 was absent but was increased significantly when S9 was present. These latter results suggested that caution should be used in estimating mutagenicity (or other toxic effects) of complex mixtures from the specific activity of individual components.

Mitchell et al. (1981) reported mutagenic activity of DPM extracts of diesel emissions in the mouse lymphoma L5178Y mutation assay. Positive results were seen both with and without

S9 activation in extracts from several different vehicles, with mutagenic activity only slightly lower in the presence of S9. These findings have been confirmed in a number of other mammalian cell systems using several different genetic markers. Casto et al. (1981), Chescheir et al. (1981), Li and Royer (1982), and Brooks et al. (1984) all reported positive responses at the HGPRT locus in Chinese hamster ovary (CHO) cells. Morimoto et al. (1986) used the APRT and Oua^r loci in CHO cells; Curren et al. (1981) used Oua^r in BALB/c 3T3 cells. In all of these studies, mutagenic activity was observed without S9 activation. Liber et al. (1981) used the thymidine kinase (TK) locus in the TK6 human lymphoblast cell line and observed induced mutagenesis only in the presence of rat liver S9 when testing a methylene chloride extract of diesel exhaust. Barfknecht et al. (1982) also used the TK6 assay to identify some of the chemicals responsible for this activation-dependent mutagenicity. They suggested that fluoranthene, 1-methylphenanthrene, and 9-methylphenanthrene could account for more than 40% of the observed activity.

Morimoto et al. (1986) injected DPM extracts (250 to 4000 mg/kg) into pregnant Syrian hamsters and measured mutations at the APRT locus in embryo cells cultivated 11 days after i.p. injection. Neutral fractions from both light-duty (LD) and HD tar samples resulted in increased mutation frequency at 2000 and 4000 mg/kg. Belisario et al. (1984) applied the Ames test to urine from Sprague-Dawley rats exposed to single applications of DPM administered by gastric intubation, i.p. injection, or s.c. gelatin capsules. In all cases, dose-related increases were seen in TA98 (without and with S9) from urine concentrates taken 24 h after particle administration. Urine from Swiss mice exposed by inhalation to filtered exhaust (particle concentration 6 to 7 mg/m^3) for 7 weeks (Pereira et al., 1981a) or Fischer 344 rats exposed to DPM (2 mg/m^3) for 3 months to 2 years was negative in Salmonella strains.

Schuler and Niemeier (1981) exposed Drosophila males in a stainless steel chamber connected to the 3 m^3 chamber used for the chronic animal studies at EPA (see Hinners et al., 1980 for details). Flies were exposed for 8 h and mated to untreated females 2 days later. Although the frequency of sex-linked recessive lethals from treated males was not different from that of controls, the limited sample size precluded detecting less than a threefold increase over controls. The authors noted that, because there were no signs of toxicity, the flies might tolerate exposures to higher concentrations for longer time periods.

Driscoll et al. (1996) exposed Fischer 344 male rats to aerosols of carbon black (1.1, 7.1, and 52.8 mg/m^3) or air for 13 weeks (6 h/day, 5 days/week) and measured *hprt* mutations in alveolar type II cells in animals immediately after exposure and at 12 and 32 weeks after the end of exposure. Both the two higher concentrations resulted in significant increases in mutant frequency. While the mutant frequency from the 7.1 mg/m^3 group returned to control levels by 12 weeks, the mutant frequency of the high exposure group was still higher than controls even after 32 weeks. Carbon black particles have very little adsorbed PAHs, hence a direct chemically-

induced mechanism is highly unlikely. The authors suggested that the likely explanation for the observed increases was persistent pulmonary inflamation and hyperplasia.

Specific-locus mutations were not induced in (C3H × 101)F_1 male mice exposed to diesel exhaust 8 h/day, 7 days/week for either 5 or 10 weeks (Russell et al., 1980). The exhaust was a 1:18 dilution and the average particle concentration was 6 mg/m^3. After exposure, males were mated to T-stock females and matings continued for the reproductive life of the males. The results were unequivocally negative; no mutants were detected in 10,635 progeny derived from postspermatogonial cells or in 27,917 progeny derived from spermatogonial cells.

Hou et al. (1995) measured DNA adducts and *hprt* mutations in 47 bus maintenance workers and 22 control individuals. All were nonsmoking men from garages in the Stockholm area and the exposed group consisted of 16 garage workers, 25 mechanics, and 6 other garage workers. There were no exposure data, but the three groups were considered to be of higher to lower exposure to diesel engine exhaust. Levels of DNA adducts determined by ^{32}P-postlabeling were significantly higher in workers than in controls (3.2 versus 2.3×10^{-8}), but *hprt* mutant frequencies were not different (8.6 versus 8.4×10^{-6}). Both adduct level and mutagenicity were highest among the 16 most exposed workers and mutant frequency was significantly correlated with adduct level. All individuals were genotyped for glutathione transferase GSTM1 and aromatic amino transferase NAT2 polymorphism. Neither GSTM1 nulls nor NAT2 slow acetylators exhibited effects on either DNA adducts or *hprt* mutant frequencies.

4.2. CHROMOSOME EFFECTS

Mitchell et al. (1981) and Brooks et al. (1984) reported increases in sister chromatid exchanges (SCE) in CHO cells exposed to DPM extracts of emissions from both LD and HD diesel engines. Morimoto et al. (1986) observed increased SCE from both LD and HD DPM extracts in PAH-stimulated human lymphocyte cultures. Tucker et al. (1986) exposed human peripheral lymphocyte cultures from four donors to direct diesel exhaust for up to 3 h. Exhaust was cooled by pumping through a plastic tube about 20 feet long; airflow was 1.5 L/min. Samples were taken at 16, 48, and 160 min of exposure. Cell cycle delay was observed in all cultures; significantly increased SCE levels were reported for two of the four cultures. Structural chromosome aberrations were induced in CHO cells by DPM extracts from a Nissan diesel engine (Lewtas, 1983) but not by similar extracts from an Oldsmobile diesel engine (Brooks et al., 1984).

Gu et al. (1992) reported that DEP dispersed in an aqueous mixture containing dipalmitoyl lecithin (DPL), a component of pulmonary surfactant or extracted with dichloromethane (DCM) induced similar responses in micronucleus tests in Chinese hamster V79 and CHO cell cultures. After the samples were separated into supernatant and sediment fractions, mutagenic activity was confined to the sediment fraction of the DPL sample and the supernatant of the DCM sample.

These findings suggest that the mutagenic activity of DEP inhaled into the lungs could be made bioavailable through solubilization and dispersion nature of pulmonary surfactants, but the application of these in vitro findings to conditions in the human lung remains to be studied.

Pereira et al. (1981a) exposed female Swiss mice to diesel exhaust 8 h/day, 5 days/week for 1, 3, and 7 weeks. The incidence of micronuclei and structural aberrations was similar in bone marrow cells of both control and exposed mice. Increased incidences of micronuclei, but not SCE, were observed in bone marrow cells of male Chinese hamsters after 6 months of exposure to diesel exhaust (Pereira et al., 1981b).

Guerrero et al. (1981) observed a linear concentration-related increase in SCE in lung cells cultured after intratracheal instillation of DPM at doses up to 20 mg/hamster. However, they did not observe any increase in SCE after 3 months of inhalation exposure to diesel exhaust particles (6 mg/m^3).

Pereira et al. (1982) measured SCE in embryonic liver cells of Syrian hamsters. Pregnant females were exposed to diesel exhaust (containing about 12 mg/m^3 particles) from days 5 to 13 of gestation or injected intraperitoneally with diesel particles or particle extracts on gestational day 13 (18 h before sacrifice). Neither the incidence of SCE nor mitotic index was affected by exposure to diesel exhaust. The injection of DPM extracts, but not DPM, resulted in a dose-related increase in SCE; however, the toxicity of the DPM was about twofold greater than the DPM extract.

In the only studies with mammalian germ cells, Russell et al. (1980) reported no increase in either dominant lethals or heritable translocations in males of T-stock mice exposed by inhalation to diesel emissions. In the dominant lethal test, T-stock males were exposed for 7.5 weeks and immediately mated to females of different genetic backgrounds (T-stock; [C3H × 101]; [C3H × C57BL/6]; [SEC × C57BL/6]). There were no differences from controls in any of the parameters measured in this assay. For heritable translocation analysis, T-stock males were exposed for 4.5 weeks and mated to (SEC × C57BL/6) females, and the F_1 males were tested for the presence of heritable translocations. Although no translocations were detected among 358 progeny tested, the historical control incidence is less than 1/1,000.

4.3. OTHER GENOTOXIC EFFECTS

Pereira et al. (1981b) exposed male strain A mice to diesel exhaust emissions for 31 or 39 weeks using the same exposure regimen noted in the previous section. Analyses of caudal sperm for sperm-head abnormalities were conducted independently in three separate laboratories. Although the incidence of sperm abnormalities was not significantly above controls in any of the three laboratories, there were extremely large differences in scoring (control values were 9.2%, 14.9%, and 27.8% in the three laboratories). Conversely, male Chinese hamsters exposed for 6

months (Pereira et al., 1981c) exhibited almost a threefold increase in sperm-head abnormalities. It is noted that the control incidence in the Chinese hamsters was less than 0.5%. Hence, it is not clear whether the differing responses reflect true species differences or experimental artifacts.

4.4. SUMMARY

Extensive studies with Salmonella have unequivocally demonstrated mutagenic activity in both particulate and gaseous fractions of diesel exhaust. In most of the studies using Salmonella, DPM extracts and individual nitropyrenes exhibited the strongest responses in strain TA98 when no exogenous activation was provided. Gaseous fractions reportedly showed greater response in TA100, whereas benzo[a]pyrene and other unsubstituted PAHs are mutagenic only in the presence of S9 fractions. The induction of gene mutations has been reported in several in vitro mammalian cell lines after exposure to extracts of DPM. Note that only the TK6 human cell line did not give a positive response to DPM extracts in the absence of S9 activation. Mutagenic activity was recovered in urine from animals treated with DPM by gastric intubation and i.p. and s.c. implants, but not by inhalation of DPM or diluted diesel exhaust. Dilutions of whole diesel exhaust did not induce sex-linked recessive lethals in Drosophila or specific-locus mutations in male mouse germ cells.

Structural chromosome aberrations and SCE in mammalian cells have been induced by particles and extracts. Whole exhaust induced micronuclei but not SCE or structural aberrations in bone marrow of male Chinese hamsters exposed to whole diesel emissions for 6 months. In a shorter exposure (7 weeks), neither micronuclei nor structural aberrations were increased in bone marrow of female Swiss mice. Likewise, whole diesel exhaust did not induce dominant lethals or heritable translocations in male mice exposed for 7.5 and 4.5 weeks, respectively.

Application of mutagenicity data to the question of the potential carcinogenicity of diesel emissions is based on the premise that genetic alterations are found in all cancers and that several of the chemicals found in diesel emissions possess mutagenic activity in a variety of genetic assays. These genetic alterations can be produce by gene mutations, deletions, translocations, aneuploidy, or amplification of genes, hence no single genotoxicity assay should be expected to either qualitatively or quantitatively predict rodent carcinogenicity. With diesel emissions or other mixtures, additional complications arise because of the complexity of the material being tested. Exercises that combined the Salmonella mutagenic potency with the total concentration of mutagenic chemicals deposited in the lungs could not account for the observed tumor incidence in exposed rats (Rosenkranz, 1993,Goldstein, et al. 1998). Additionally, it appears that the some of constituents responsible for the mutational increases observed in bacteria are different from those responsible for the observed increases in CHO cells (Li and Dutcher, 1983) or in human hepatoma-derived cells (Eddy et al., 1986).

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